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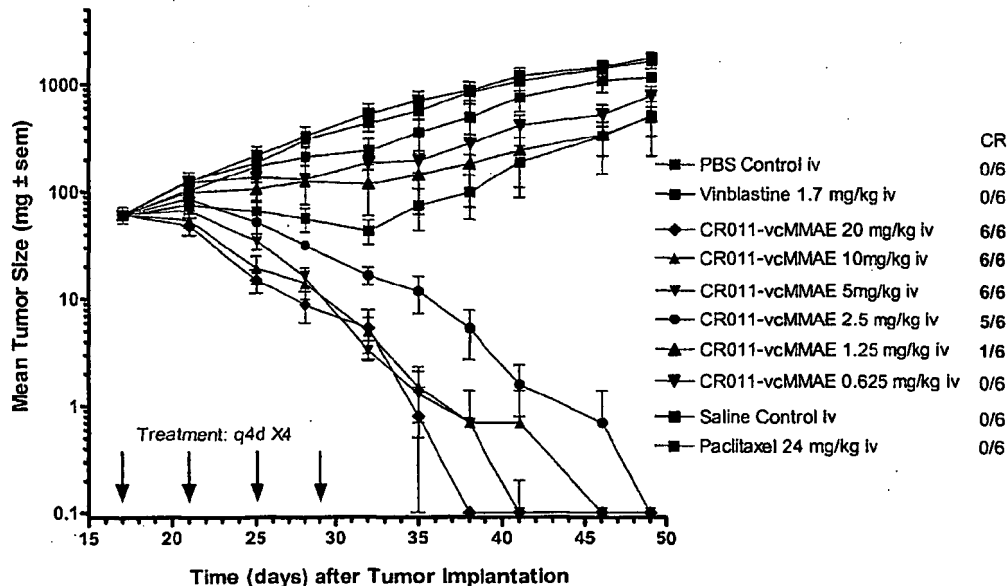
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(54) Title: ANTIBODIES DIRECTED TO GPNMB AND USES THEREOF



(57) Abstract: The present invention provides fully human monoclonal antibodies that specifically bind to GPNMB, and uses thereof. Nucleotide sequences encoding, and amino acid sequences comprising, heavy and light chain immunoglobulin molecules, particularly sequences corresponding to contiguous heavy and light chain sequences spanning the framework regions and/or complementarity determining regions (CDRs) are provided. The present invention also provides immunoconjugates comprising anti-GPNMB antibodies and methods of using such immunoconjugates. The present invention further provides bispecific antibodies comprising an anti-GPNMB antibody component and an anti-CD3 component, and methods of using such bispecific antibodies.

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ANTIBODIES DIRECTED TO GPNMB AND USES THEREOF

FIELD OF THE INVENTION

The present invention relates to antibodies with specificity to GPNMB, and uses of such antibodies. In particular, the present invention provides fully human monoclonal antibodies that specifically bind to GPNMB, and uses thereof. Nucleotide sequences encoding, and amino acid sequences comprising, heavy and light chain immunoglobulin molecules, particularly sequences corresponding to contiguous heavy and light chain sequences spanning the framework regions and/or complementarity determining regions (CDRs) are provided. The present invention also provides immunoconjugates comprising anti-GPNMB antibodies and methods of using such immunoconjugates. The present invention further provides bi-specific antibodies comprising an anti-GPNMB antibody component and an anti-CD3 component, and methods of using such bispecific antibodies.

BACKGROUND OF THE INVENTION

GPNMB

A putative transmembrane glycoprotein called "*nmb*" (Acc. No. X76534 EMBL), referred to herein as GPNMB, was identified and described by Weterman *et al.*, (Int J Cancer 60:73-81, 1995) as differentially expressed in low-metastatic human melanoma cancer cell lines and xenografts, compared to a more aggressive melanoma cell line. GPNMB shares 33% identity with the precursor of pMel17 melanocyte-specific protein (Kwon *et al.*, 1991, PNAS 88:9228-9232). GPNMB is 71% homologous to a dendritic cell-associated transmembrane protein, DC-HIL (Shikano *et al.*, 2001 Biol. Chem. 276:8125-8134). GPNMB is also known as the hematopoietic growth factor inducible neurokinin-1 protein HGFIN (Bandari *et al.*, Reg. Peptides 111:169-178) and the bone-related gene osteoactivin (Owen *et al.* Crit Rev Eukaryot Gene Expr 2003, 13(2-4):205-220)

It was also reported that *nmb* could reduce the metastatic potential of a highly metastatic *nmb*-negative melanoma cell line (Weterman, 1995). GPNMB was considered a candidate glioblastoma tumor marker after public database mining and expression profiling (Loging *et al.*, 2000, Genome Research 10:1393-1402). This gene was found overexpressed in lung tumors (US Patent Publication No. US20030064947), as well as breast, rectal and colon cancers (US Patent Publication No. US2003100720). NCBI SAGE data also shows overexpression of this gene in stomach and pancreatic carcinoma. The mouse ortholog has been shown to be highly upregulated in a neural stem cell line NSC,

derived from the TSC2 knockout model for Tuberous Sclerosis Complex Syndrome (International Publication No. WO 2003/080856).

Antibodies

Antibodies, also known as immunoglobulins, are typically tetrameric glycosylated proteins composed of two light (L) chains (about 25 kDa) and two heavy (H) chains (about 50-70 kDa). The amino-terminal portion of each chain includes a variable domain of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of the L and H chain has one and three or four constant domains, respectively that are primarily responsible for effector function. There are two types of human L chains, classified as kappa and lambda. H chains are classified as mu, delta, gamma, alpha, or epsilon based upon the constant domain amino acid sequence, defining the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Isotypes may be further divided into subclasses *e.g.* IgG₁, IgG₂, IgG₃, IgG₄.

Immunoglobulins can be produced naturally *in vivo* by B lymphocytes. Each clone of B cells produces antibody with an antigen receptor having a unique prospective antigen binding structure. The repertoire of antigen receptors, approximately 10^7 possibilities, exists *in vivo* prior to antigen stimulation. This diversity is produced by somatic recombination, *i.e.*, the joining of different antibody gene segments. Immunoglobulin H chain, kappa L chain and lambda L chain are encoded by three separate genetic loci and each locus has multiple copies of at least 3 types of gene segments encoding variable (V), constant (C) and joining (J) regions, the heavy chain gene also includes a diversity (D) region. The selection of specific V, C and J regions (and D for the heavy chain) from amongst the various gene segments available (45 heavy chain V; 35 kappa V; 23 heavy chain D; 6 heavy chain J; 5 kappa J) generates approximately 10^{11} possible specificities of germline sequences exhibited in B cells. The joining of V, C and J regions can result in the loss or addition of residues at the junctions. The L and H chain V region of human antibodies consists of relatively conserved framework regions (FR) that form a scaffold for three hypervariable regions also known as complementary determining regions (CDR). From the amino terminus of either the heavy or light chain, the V domain is made up of FR and CDR regions in the following order: FR1-CDR1-FR2-CDR2-FR3. Joining of the V domain with a D (heavy chain only) and J domain adds CDR3-FR4. The CDRs are generally responsible for antigen binding.

The specificity of monoclonal antibodies have made them attractive agents for targeting cancer in vivo with the hopes of eradicating disease while sparing normal tissue. The approach, which initially utilized mouse monoclonal antibodies has encountered limitations to potential effectiveness such as immunogenicity; inefficient effector functions and short half-life in vivo. Technologies were developed for: chimeric antibodies which sought to utilize the antigen binding variable domains of mouse monoclonal antibodies combined with the constant regions of human antibodies (*Boulianne, et al. 1984 Nature 312:643-646; Morrison et al, 1984 PNAS USA 81:6851-6855*); humanized antibodies which grafted antigen binding complementary determining regions (CDRs) from mouse antibodies to human immunoglobulin (*Jones, et al, 1986 Nature 321: 522-525; Riechmann, et al, 1988 Nature 332:323-327; Verhoeyen, et al, 1988 Science 239:1534-1536; Vaughan, et al, 1998 Nature Biotechnol. 16:535-539*); and phage display libraries of single chain scFvs or Fab fragments of antibodies (*de Haard, et al, 1999 J Biol. Chem. 274: 18218-18230; Knappik, et al, 2000 J. Mol. Biol. 296:57-86; Sheets, et al, 1998 PNAS USA 95:6157-6162; Vaughan, et al, 1994 Nature Biotechnol 14:309-314, 1996; Griffiths et al EMBO J. 13:3245-3260*). Additionally, transgenic animals having human immunoglobulin genes and nonfunctional endogenous genes have been developed for immunization and production of fully human monoclonal antibodies (*Fishwild, et al, 1996 Nature Biotechnol 14:845-851; Mendez, et al, 1997 Nature Genet. 15:146-156; Nicholson, et al, 1999 J. Immunol 163, 6898-6906*).

Single Chain Antibodies: Single chain Fv antibodies (scFvs) were first described in the late 1980's (*Bird et al., Science 242:423-426 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988)*). A polypeptide linker, typically ranging in length from 5 to 27 amino acid residues, is used to join the C-terminus of the variable light chain domain (V_L) to the N-terminus of the variable heavy chain domain (V_H). Alternatively, the linker joins the C-terminus of the V_H to the N-terminus of the V_L . Both formats (V_L - V_H and V_H - V_L) have been used successfully in the literature. The most common linker used in the literature is the $(Gly_4Ser)_3$ 15 amino acid linker, however there are several other linkers that have been utilized, including a 25 amino acid linker called 205C (*Pantoliano et al., Biochemistry 30:10117-10125 (1991)*). Single chain antibodies are currently in the clinic; one of the most advanced is h5G1.1 or Pexelizumab. This scFv is specific for human C5 complement and is being used in clinical trials for cardiac patients undergoing cardiopulmonary bypass surgery (*Shernan et al., Ann. Thorac Surg. 77:942-949 (2004)*).

Bispecific Antibodies (bi-Abs): An area of mAb research where considerable progress has been made is in the development of bispecific antibodies (biAbs). There are distinct advantages to developing therapeutic antibody molecules with dual specificity. For example, biAbs can serve as mediators to target immune effector cells such as CTLs to unwanted cells (Baeuerle *et al.*, Curr. Opin. Mol. Ther. 5:413-419 (2003)). In another example, chemically linked bispecific antibodies directed against Fc gamma receptors CD16, CD64, and CD89, were significantly more effective in vitro than conventional IgG antibodies (Peipp and Valerius, Biochem. Soc. Trans. 30:507-511 (2002)). One of the challenges in developing biAbs as viable therapeutics has been producing large enough quantities of a stable moiety for clinical applications. Another challenge has been in determining the right combination of validated targets and the underlying biology that would lead to a therapeutic product. For recent reviews on the difficulties experienced with biAbs, see (Kontermann, Acta Pharmacol Sin 26:1-9 (2005); Peipp and Valerius, Soc. Trans. 30:507-511 (2002)).

Bispecific Single Chain Antibodies (bi-scFv): A notable type of biAb that can be made is a bi-specific single chain antibody or bi-scFv. For a review on the generation of bi-scFv's see (Kipriyanov and Le Gall, Curr Opin Drug Discov Devel 7:233-242 (2004)). Bi-scFvs are typically comprised of 4 variable domains, 2 heavy (V_H) and 2 light (V_L), which are derived from 2 different antibodies. The 4 domains are linked together with 3 short linkers, ranging in length from 5-27 amino acids. The biological activity of this type of antibody depends on several features concerning the construction of the molecule. For example, both the linker sequences between the antibody V domains and the order of the 4 antibody V domains themselves (for the 2 antibodies) can vary, as well as the expression system that is used; all of which can greatly affect the solubility and biological activity of the various resulting products (Kipriyanov *et al.*, J. Mol. Biol. 330:99-111 (2003); Le Gall *et al.*, Protein Eng. Des. Sel. 17:357-366 (2004); Pavlinkova *et al.*, Clin Cancer Res. 5:2613-1619 (1999)).

Cytotoxic T lymphocytes: Under normal circumstances, T cells are activated when the CD3/T cell receptor (CD3/TCR) complex binds to a relevant MHC molecule associated with a specific Ag peptide. Engagement of CD3/TCR with MHC results in intracellular signals necessary to trigger an immune response against a pathogen or tumor. Similar signals that cause T cell activation can also be achieved by antibodies that can bind certain structures of the CD3/TCR complex. In the literature, it has been shown that biAbs

recognizing both the TCR/CD3 complex and tumor associated antigen (TAA) can trigger the activation program in CTLs in the presence of target cells (Chapoval *et al.*, J. Immunol 155:1296-1303 (1995)).

Recombinant technologies are being utilized to enable further improvements upon
5 antibody molecules with the goal of enhancing *in vivo* efficacy. Such technologies provide, for example, for optimizing molecular size, affinity, pharmacokinetics, toxicity, specificity, valency, effector functions, direct and indirect arming, combination therapy, and various prodrug approaches.

It would be desirable to have an antibody suitable for *in vivo* targeting of GPNMB
10 expressing pathologies and to enable therapeutic efficacy.

SUMMARY OF THE INVENTION

The current invention provides human monoclonal antibodies that specifically bind GPNMB as well as variants, derivatives and antigen binding fragments of such antibodies.

The invention provides preferred somatic recombinations of human antibody gene
15 segments to provide specificity for GPNMB and genetically engineered anti-GPNMB antibody variants and derivatives that originate from these gene segments. In addition, the current invention provides multiple affinity matured human antibodies with binding specificity for GPNMB.

In one embodiment, the present invention provides an antibody, or binding fragment
20 thereof, that binds to GPNMB, wherein said antibody, or binding fragment thereof, neutralizes a GPNMB-induced activity, and wherein said antibody, or binding fragment thereof, cross-reacts with a fully human anti-GPNMB antibody selected from the group consisting of Mab1.2.1, Mab1.10.1, and Mab2.22.1 or an antibody in the same antigen-binding bin as fully human anti-GPNMB antibody Mab1.2.1, Mab1.10.1, or Mab2.22.1.

25 In another embodiment, the present invention provides an antibody, or binding fragment thereof, that binds to GPNMB, wherein said antibody, or binding fragment thereof, neutralizes a GPNMB-induced activity, and wherein said antibody, or binding fragment thereof, cross-reacts with a fully human anti-GPNMB antibody selected from the group consisting of Mab2.3.1 and Mab1.15.1 or an antibody in the same antigen-binding
30 bin as fully human anti-GPNMB antibody Mab2.3.1 or Mab1.15.1.

In yet another embodiment, the present invention provides an antibody, or binding fragment thereof, that binds to GPNMB, wherein said antibody, or binding fragment thereof, neutralizes a GPNMB-induced activity, and wherein said antibody, or binding

fragment thereof, cross-reacts with fully human anti-GPNMB antibody Mab2.10.1 or an antibody in the same antigen-binding bin as fully human anti-GPNMB antibody Mab2.10.1.

In one embodiment, the present invention provides naked IgG1 anti-GPNMB antibodies that have cytotoxic effect to cells overexpressing GPNMB. In a specific
5 embodiment, the present invention provides methods of treating or preventing diseases associated with overexpression of GPNMB comprising administering to a subject in need thereof a composition comprising a naked IgG1 anti-GPNMB antibody and an immunomodulator (such as, but not limited to, interferons and cytokines).

In another embodiment, the present invention provides immunoconjugates that
10 comprise an anti-GPNMB antibody or a fragment thereof, and a cytotoxic agent. In a specific embodiment, the cytotoxic agent is auristatin E (dolastatin-10) or a derivative thereof. Methods of using such immunoconjugated are also provided.

In one embodiment, the present invention provides bispecific antibodies comprising an anti-GPNMB component and an anti-CD3 antibody component, which enable the
15 cytotoxic killing of target tumor cells by T cells. In another embodiment, the present invention provides single chain Fv antibody conjugated to a cytotoxic agent. In a specific embodiment, the cytotoxic agent is auristatin E (dolastatin-10) or a derivative thereof. Methods of using such bispecific antibodies and conjugated single chain Fv antibodies are also provided.

20 Amino acid sequences for anti-GPNMB human monoclonal antibodies of the invention and nucleic acid sequences encoding them are provided.

Compositions comprising human anti-GPNMB antibodies, including therapeutic compositions comprising same, and methods of use are provided. Particularly, therapeutic immunoconjugates comprising anti-GPNMB antibodies and a cytotoxic or cytostatic agent
25 for treating GPNMB expressing cancers and other GPNMB related disorders are provided. Dosage regimens are also provided.

Additional aspects of the disclosure will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practicing the invention.

30

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Tumor growth inhibition and complete regression of SK-MEL-2 xenografts in athymic mice after treatment with 2.50 to 20 mg/kg i.v. every 4 days for 4

treatments. The responses of tumor-bearing animals to reference drugs such as vinblastine (1.7 mg/kg i.v. q4d X4) and paclitaxel (24 mg/kg i.v. q2d X4) are also shown. Control groups are treated with either phosphate-buffered saline (PBS) or physiological saline.

Figure 2: Indirect immunotoxin killing of UACC-62 melanoma cells by anti-GPNMB antibodies

Figure 3: Inhibition of colony formation of UACC-62 cells incubated with Auristatin E (AE) conjugated anti-GPNMB antibodies.

Figure 4: Tumor growth inhibition and complete regression of SK-MEL-2 xenografts in athymic mice after treatment with CR011-vcMMAE 5.0 mg/kg i.v. every 4 days for 4 treatments. The lack of responses of tumor-bearing animals to unconjugated CR011 or to free monomethylauristatin E demonstrate that the intact immunoconjugate is essential for anti-tumor effects.

Figure 5: Tumor size reduction and complete regression of SK-MEL-2 xenografts in athymic mice after treatment with 1.25 to 20 mg/kg i.v. every 4 days for 4 treatments. The responses of tumor-bearing animals to reference drugs such as Vinblastine (1.7 mg/kg i.v. q4d X4) and paclitaxel (24 mg/kg i.v. q2d X4) are also shown. Control groups are treated with either phosphate-buffered saline (PBS) or physiological saline.

Figure 6: The serum concentration-time profile of the antibody of CR011-vcMMAE after intravenous administration of 1 and 10 mg/kg in athymic mice. Detection was achieved with a sandwich ELISA assay, which employed the CR011 antigen (CG56972, GPNMB) and a horseradish peroxidase-conjugated anti-human globulin. Results shown are the serum concentrations expressed as $\mu\text{g/mL}$ (left x-axis) and micromolar concentration (right X-axis).

Figure 7: Aggregate responses, expressed as percent cures, were recorded for test animals treated with 5 different, graduated dosing intervals (*i.e.*, 0, 1, 4, 8, and 16 days between treatments). The slope of the line is not significantly different from 0 ($p < 0.2904$).

Figure 8: The proportions of complete regressors as a function of dosing interval and stratified by cumulative dose. For each group, $n = 6$ mice/group. Athymic mice bearing established SK-MEL-2 tumor implants (day 14, 80 mg) were treated i.v. with CR011-vcMMAE and the incidence of complete regressions is recorded.

Figure 9: Effects of ectopic expression of GPNMB or sensitivity to CR011-vcMMAE. HEK293 cells are transfected with empty vector (vector) or GPNMB-containing plasmid (GPNMB) as described in Materials and Methods. A. Cell lysates are prepared

from the transfected HEK293 cells and the expression of GPNMB (upper panel) or actin (lower panel) is determined by immunoblotting. Lane 1: Empty vector transfectants. Lane 2: GPNMB transfectants. **B.** Flow cytometry analysis of GPNMB expression on empty vector or GPNMB transfected cells. **C.** CR011-vcMMAE in vitro growth inhibition of transfected cells. Cells are treated with various concentrations of CR011-vcMMAE (diamonds: vector or circles: GPNMB) or IgG2-vcMMAE (triangles: vector or squares: GPNMB) for 96 hours. After a clonogenic assay, the surviving fraction is normalized to the untreated control and expressed as a percentage of the control using GraphPad Prism graphing software. Each treatment is performed in triplicate. A representative graph from two independent experiments is shown.

Figure 10: Effect of GPNMB siRNA on endogenous GPNMB expression and sensitivity to CR011-vcMMAE. SK-Mel-2 cells are transfected with 50 nM of control siRNA or siRNA targeting GPNMB. **A.** Cell lysates are prepared from the transfected SK-Mel-2 cells 2 and 4 days post-transfection and the expression of GPNMB (upper panel) or actin (lower panel) is determined by immunoblotting. Lane 1: Mock (oligofectamine) transfection. Lane 2: Control siRNA transfection. Lane 3: GPNMB siRNA transfection. **B.** Flow cytometry analysis of GPNMB expression 2 and 4 days after transfection. SK-Mel-2 cells are transfected with mock, control siRNA or GPNMB siRNA as indicated in the Materials and Methods. **C.** CR011-vcMMAE in vitro growth inhibition of mock (diamonds), control siRNA (circles) or GPNMB siRNA (triangles) transfected SK-Mel-2 cells is determined by a clonogenic assay as described in Materials and Methods. The surviving fraction is normalized to the untreated control and expressed as a percentage of control using GraphPad Prism graphing software. Each treatment is performed in triplicate. A representative experiment from two independent studies is shown.

Figure 11: FACS analysis of SK-MEL-2 with isotype control, hybridoma IgG2 (B2), recombinant IgG2 (B19) and recombinant IgG1 (B16) to CG56972/GPNMB relative to IgG2 (B2, B19) or IgG1 (Control, B16) controls.

Figure 12: (A) PBMC and mAb (IgG1) mediated ADCC of SK-MEL-2 cells. ADCC effector functions are measured as described above at 2, 5 and 10 $\mu\text{g}/200\ \mu\text{l}$ using target:effector ratios of 10, 30, 60 and 100 as indicated. (B) PBMC and mAb (IgG2) do not cause ADCC to SK-MEL-2 cells. ADCC effector functions are measured as described above at 0, 2, 5 and 10 $\mu\text{g}/200\ \mu\text{l}$ using target: effector ratios of 10, 30, 60 and 100 as indicated.

Figure 13: Expression of CG56972 in human cancer cell lines and tissues. RTQ PCR analysis of (A) human brain cancer cell lines or (B) human brain cancer glioma and medulloblastoma biopsies. (C) Microarray analysis of CG56972 expression in human brain cancer and oligodendroglioma tissues. Tissues or cell lines are harvested, mRNA prepared
 5 and RTQ PCR or CuraChip analysis performed as described in Materials and Methods.

Figure 14: FACS analysis of cell surface binding of CR011 mAb to CG56972. SK-MEL-2, XF-498, U-118-MG, SNB-78, SF-539 and SF-268 cells are labeled with a saturating concentration (10 µg/mL) of CR011 mAb or control IgG2. Bound mAb is detected by flow cytometry with PE-conjugated goat-anti-human secondary antibody as
 10 described in Materials and Methods. GM: Geometric mean. The SF-268 cell line is CG56972 transcript negative and used as a negative control.

Figure 15: Immunoblot analysis of CG5672 expression in human brain cancer cell lines. Cell lysates are resolved on Tris-glycine gels and transferred to membranes. Immunoblot analysis is carried out with a polyclonal antibody to CG56972 followed by
 15 enhanced chemiluminescence detection as described in Materials and Methods. Arrowheads indicate the relative mobility of the p100 and 120 CG56972 species. The SF-268 cell line is CG56972 transcript negative and used as a negative control.

Figure 16: CR011-vcMMAE in vitro growth inhibition of astocytoma/glioblastoma cell growth. XF-498, SNB-78, U-118-MG, SF-539, LOXIMVI and SF-268 cells are
 20 incubated with the indicated concentration of CR011-vcMMAE. Cells are also incubated with control PK16.3 mAb (data shown in Table I) as described in the Materials and Methods. Cell growth was determined by clonogenic assay. The surviving colonies are counted and plotted using GraphPad Prism graphing software. The experiment is performed in triplicate wells and repeated twice. vA representative experiment is shown. IC50s for
 25 cell killing is presented in ng/mL concentrations. The LOXIMVI and SF-268 cell lines are CG56972 transcript negative and used as negative controls.

Figure 17: Development of CR011 Engineered Antibodies. Four antibody variable (V) domains (shown in C for the bi-scFv) are derived from the light and heavy chain variable domains (V_L and V_H) making up the antigen binding sites of CR011 and anti-CD3 whole
 30 IgGs. The middle linker joining the 2 individual scFv components together (shown in dashed line) may play a key role in determining the resulting activity of each of the scFv components, including the effective cytolytic activity provided by the cytotoxic T cells engaged by the anti-CD3 scFv component of the bi-scFv.

Figure 18: A. ELISA results for CR011 scFv (squares) and CR011 x anti-CD3 (L4-L2-L4 linker set) bi-scFv (diamonds). Both engineered CR011 antibodies bound to the GPNMB target. B. Western blotting of 2 of the CR011 engineered antibody products (arrows). Clone 16 corresponded to the CHOK1 line expressing CR011 scFv (monomer), while clone 17 corresponded to the CHOK1 line expressing CR011 x anti-CD3 (L4-L2-L4 linker set) bi-scFv (dimer). Clones 16 and 17 are used to produce the engineered antibody products.

Figure 19: Flow cytometry analysis of binding of CR011 scFv and CR011 x anti-CD3 (L4-L2-L4 linker set) bi-scFv products to native GPNMB protein expressed on the cell surface of target cells. Human T cells are used as a source of CD3, while SK-Mel-5 cells are used as a source of GPNMB.

Figure 20: Cytotoxicity analysis showed that purified CR011 x anti-CD3 (L4-L2-L4 linker set) bi-scFv, but not CR011 scFv, causes killing of GPNMB positive SK-Mel-5 tumor cells by T lymphocytes.

Figure 21: The chemical structure of Maleimidocaproyl-Valine-Citrullin-Monomethyl-Auristatin E (vcMMAE).

Figure 22: Disulfides on CR011 antibody are gently reduced in the presence of TCEP to generate ~4 thiols per Ab. vcMMAE is then added to antibody solution. Nucleophilic attack of thiolates on maleimide-groups results in a stable thioester linkage. The resulting conjugate is purified from the mixture.

Figure 23: Reaction of vcMMAE with NAcCys at pH 7.0 and pH 9.0 in the presence or absence of TCEP. 1A: VCMMAE converts fully into NAcCys-adduct following a incubation in phosphate pH 7 buffer. B-E: Appearance of a side product in a course of incubation of vcMMAE in borate buffer. F-I: Appearance of side products in borate pH 9 and in the presence of TCEP.

Figure 24: LCMS identification of the side product with retention time of 9.2 min not capable of reaction with cystein and therefore, not capable of conjugation to CR011.

Figure 25: Kinetics of the formation of NAcCys-vcMMAE and of the side product (succinimidyl-vcMMAE) following incubation in borate pH 9.0 buffer in the presence or absence of TCEP.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "antibody" refers to an immunoglobulin or a fragment or a derivative thereof, and encompasses any polypeptide comprising an antigen-binding site, regardless whether it is produced in vitro or in vivo. The term includes, but is not limited to, polyclonal, monoclonal, monospecific, polyspecific, non-specific, humanized, single-chain, chimeric, synthetic, recombinant, hybrid, mutated, engineered, and grafted antibodies. Unless otherwise modified by the term "intact," as in "intact antibodies," for the purposes of this disclosure, the term "antibody" also includes antibody fragments such as Fab, F(ab')₂, Fv, scFv, bi-scFv, bi-Ab, Fd, dAb, and other antibody fragments that retain antigen-binding function, *i.e.*, the ability to bind GPNMB specifically. Typically, such fragments would comprise an antigen-binding domain.

As used herein, the terms "antigen-binding domain," "antigen-binding fragment," and "binding fragment" refer to a part of an antibody molecule that comprises amino acids responsible for the specific binding between the antibody and the antigen. In instances, where an antigen is large, the antigen-binding domain may only bind to a part of the antigen. A portion of the antigen molecule that is responsible for specific interactions with the antigen-binding domain is referred to as "epitope" or "antigenic determinant."

An antigen-binding domain typically comprises an antibody light chain variable region (V_L) and an antibody heavy chain variable region (V_H), however, it does not necessarily have to comprise both. For example, a so-called Fd antibody fragment consists only of a V_H domain, but still retains some antigen-binding function of the intact antibody.

As used herein, the term "repertoire" refers to a genetically diverse collection of nucleotides derived wholly or partially from sequences that encode expressed immunoglobulins. The sequences are generated by in vivo rearrangement of, *e.g.*, V, D, and J segments for H chains and, *e.g.*, V and J segment for L chains. Alternatively, the sequences may be generated from a cell line by in vitro stimulation, in response to which the rearrangement occurs. Alternatively, part or all of the sequences may be obtained by combining, *e.g.*, unrearranged V segments with D and J segments, by nucleotide synthesis, randomised mutagenesis, and other methods, *e.g.*, as disclosed in U.S. Pat. No. 5,565,332.

As used herein, the terms "specific interaction" and "specific binding" refer to two molecules forming a complex that is relatively stable under physiologic conditions. Specific binding is characterized by a high affinity and a low to moderate capacity as distinguished

from nonspecific binding which usually has a low affinity with a moderate to high capacity. Typically, binding is considered specific when the affinity constant K_A is higher than $10^6 M^{-1}$, or more preferably higher than $10^8 M^{-1}$. If necessary, non-specific binding can be reduced without substantially affecting specific binding by varying the binding conditions. The appropriate binding conditions such as concentration of antibodies, ionic strength of the solution, temperature, time allowed for binding, concentration of a blocking agent (e.g., serum albumin, milk casein), etc., may be optimized by a skilled artisan using routine techniques.

As used herein, the term "substantially as set out" refers that the relevant CDR, V_H , or V_L domain of the invention will be either identical to or have only insubstantial differences in the specified regions (e.g., a CDR), the sequence of which is set out. Insubstantial differences include minor amino acid changes, such as substitutions of 1 or 2 out of any 5 amino acids in the sequence of a specified region.

As used herein, the term "CR011" refers to a fully human monoclonal antibody that specifically binds to GPNMB. In some embodiments, CR011 refers to those antibodies that are identified in Tables 2A-2D of the present application. In some embodiments, CR011 refers to Mab 1.15.1 as described in the instant invention.

The terms "GPNMB" and "CG56972" are used interchangeably herein. As used herein, the terms "GPNMB" or "CG56972" refer to a transmembrane glycoprotein that has an amino acid sequence as set forth in SEQ ID NO: 289, an analog, derivative or a fragment thereof, or a fusion protein comprising GPNMB, an analog, derivative or a fragment thereof. In certain embodiments, the term "GPNMB" refers to the mature, processed form of GPNMB. In other embodiments, the term "GPNMB" refers to the extracellular domain of GPNMB.

As used herein, the term "GPNMB activity" refers to one or more activities associated with GPNMB. To "modulate" GPNMB activity is to alter the baseline results observed with, and that can be attributed to GPNMB. To "neutralize" GPNMB is to cancel one or more effects, e.g. activity observed with, and that can be attributed to GPNMB.

As used herein, the term "isolated" refers to a molecule that is substantially free of its natural environment. For instance, an isolated protein is substantially free of cellular material or other proteins from the cell or tissue source from which it is derived. The term "isolated" also refers to preparations where the isolated protein is sufficiently pure to be administered as a pharmaceutical composition, or at least 70-80% (w/w) pure, more

preferably, at least 80-90% (w/w) pure, even more preferably, 90-95% pure; and, most preferably, at least 95%, 96%, 97%, 98%, 99%, or 100% (w/w) pure.

As used herein, the term "inhibit" or "inhibition of" refers to reducing by a measurable amount, or to prevent entirely.

5 As used herein, the term "Cytotoxic effect" in reference to the effect of an agent on a cell, means killing of the cell. "Cytostatic effect" refers to an inhibition of cell proliferation. A "cytotoxic agent" refers an agent that has a cytotoxic or cytostatic effect on a cell, thereby depleting or inhibiting the growth of, respectively, cells within a cell population.

10 As used herein, the terms "prevent," "preventing," and "prevention" refer to the inhibition of the development or onset of a disorder associated with aberrant expression and/or activity of GPNMB (*e.g.*, cancer) or the prevention of the recurrence, onset, or development of one or more symptoms of a disorder associated with aberrant expression and/or activity of GPNMB (*e.g.*, cancer) in a subject resulting from the administration of a therapy or the administration of a combination of therapies.

15 As used herein, the term "effective amount" refers to a dosage or amount that is sufficient to reduce the activity of GPNMB to result in amelioration of symptoms in a patient or to achieve a desired biological outcome.

20 As used herein, the term "prophylactically effective amount" refers to the amount of a therapy which is sufficient to result in the prevention of the development, recurrence, or onset of a disorder associated with aberrant expression and/or activity of GPNMB (*e.g.*, cancer) or one or more symptoms thereof, or to enhance or improve the prophylactic effect(s) of another therapy.

As used herein, a "protocol" includes dosing schedules and dosing regimens. The protocols herein are methods of use and include prophylactic and therapeutic protocols.

25 As used herein, the terms "subject" and "patient" are used interchangeably. As used herein, the terms "subject" and "subjects" refer to an animal, preferably a mammal including a non-primate (*e.g.*, a cow, pig, horse, cat, dog, rat, and mouse) and a primate (*e.g.*, a monkey, such as a cynomolgous monkey, chimpanzee, and a human), and more preferably a human.

30 As used herein, the terms "therapeutic agent" and "therapeutic agents" refer to an agent that can be used in the prevention, treatment, management, or amelioration of a disorder associated with aberrant expression and/or activity of GPNMB (*e.g.*, cancer) or one or more symptoms thereof. In certain embodiments, the term "therapeutic agent" refers to

an antibody that immunospecifically binds to GPNMB. In certain other embodiments, the term "therapeutic agent" refers an agent other than an antibody that immunospecifically binds to GPNMB.

As used herein, the terms "therapies" and "therapy" can refer to any protocol(s),
5 method(s), and/or agent(s) that can be used in the prevention, treatment, management, or amelioration of a disorder associated with aberrant expression and/or activity of GPNMB (e.g., cancer) or one or more symptoms thereof. In certain embodiments, the terms "therapies" and "therapy" refer to anti-cancer therapy, biological therapy, supportive therapy, and/or other therapies useful in treatment, management, prevention, or amelioration
10 of cancer or one or more symptoms thereof known to one of skill in the art such as medical personnel.

As used herein, the terms "treat," "treatment," and "treating" refer to the eradication, removal, modification, or control of primary, regional, or metastatic cancer tissue, or the reduction or amelioration of the progression, severity, and/or duration of a disorder
15 associated with aberrant expression and/or activity of GPNMB or amelioration of one or more symptoms thereof resulting from the administration of one or more therapies. In certain embodiments, such terms in the context of cancer refer to a reduction in the growth of cancerous cells, a decrease in number of cancerous cells and/or a reduction in the growth, formation and/or volume of a tumor. In other embodiments, such terms refer to the
20 minimizing or delay of the spread of cancer resulting from the administration of one or more therapies to a subject with such a disease. Treatment can include, for example, a decrease in the severity of a symptom, the number of symptoms, or frequency of relapse.

Unless otherwise defined, scientific and technical terms used in connection with the invention described herein shall have the meanings that are commonly understood by those
25 of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well known and commonly used in the art. Standard techniques
30 are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures

are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. (See e.g., *Sambrook et al. Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1989). The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

10 The current invention provides germline human antibody heavy chain V, D, J combinations and light chain V, J combinations including nucleotide and amino acid sequence of the V_H and V_L domain FR and CDR regions with specificity for GPNMB.

 Upon exposure to antigen, those B cells with antigen binding specificity based on germline sequences are activated, proliferate, and differentiate to produce immunoglobulins of different isotypes as well as undergo somatic mutation and/or affinity maturation to produce immunoglobulins of higher affinity for the antigen. The current invention provides the nucleotide and amino acid sequence of such affinity matured V domain FR and CDR regions having specificity to GPNMB.

 Fab type antibody fragments containing the antigen binding portion of the antibody molecule may consist of the L chain covalently linked by a disulfide bond to a portion of the H chain which has the V domain and first constant domain. Single chain Fv antibody fragment (scFv) has the H variable domain linked to the L variable domain by a polypeptide linker. The invention provides antibody fragments such as Fab and scFv molecules having sequences derived from germline or affinity matured V domains of antibodies binding specifically to GPNMB.

 A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments (see, e.g., Songsivilai & Lachmann, 1990 Clin. Exp. Immunol. 79: 315-321; Kostelny *et al.*, 1992 J. Immunol. 148:1547-1553). Bispecific antibodies do not exist in the form of fragments having a single binding site (e.g., Fab, Fab', and Fv).

 It will be appreciated that such bifunctional or bispecific antibodies are contemplated and encompassed by the invention. A bispecific single chain antibody with

specificity to GPNMB and to the CD3 antigen on cytotoxic T lymphocytes can be used to direct these T cells to tumor cells expressing GPNMB and cause apoptosis and eradication of the tumor. Bispecific scFv constructs for this purpose are described herein. The scFv components specific for GPNMB can be derived from anti-GPNMB antibodies described
 5 herein. In some embodiments, the anti-GPNMB antibody components disclosed herein can be used to generate a biologically active scFv directed against GPNMB. The anti-CD3 scFv component of the therapeutic bispecific scFv was derived from a sequence deposited in Genbank (accession number CAE85148). Alternative antibodies known to target CD3 or other T cell antigens may similarly be effective in treating malignancies when coupled with
 10 anti-GPNMB, whether on a single-chain backbone or a full IgG.

GPNMB binding human antibodies may include H or L constant domains including L kappa or lambda constant regions, or any isotype H constant domain. In one embodiment of the invention, a human antibody with binding specificity to GPNMB contains germline sequences such as the heavy chain V regions: VH1-2 (SEQ ID NO: 308), VH2-5 (SEQ ID
 15 NO: 360), VH3-11 (SEQ ID NO: 361), VH3-21 (SEQ ID NO: 362), VH3-30 (SEQ ID NO: 363), VH3-33 (SEQ ID NO: 364), VH4-31 (SEQ ID NO: 365), VH4-59 (SEQ ID NO: 366) or VH5-51 (SEQ ID NO: 367); the heavy chain D region: D1-20 (amino acid sequences translated by SEQ ID NO: 375), D1-26 (amino acid sequences translated by SEQ
 20 ID NO: 376), D3-10 (amino acid sequences translated by SEQ ID NO: 377), D3-16 (amino acid sequences translated by SEQ ID NO: 378), D3-22 (amino acid sequences translated by SEQ ID NO: 379), D3-9 (amino acid sequences translated by SEQ ID NO: 380), D4-17 (amino acid sequences translated by SEQ ID NO: 381), D5-24 (amino acid sequences translated by SEQ ID NO: 382), D6-13 (amino acid sequences translated by SEQ ID
 25 NO: 383), or D6-19 (amino acid sequences translated by SEQ ID NO: 384); the heavy chain J region: JH3b (SEQ ID NO: 385), JH4b (SEQ ID NO: 386), JH5b (SEQ ID NO: 387) or JH6b (SEQ ID NO: 388); the light chain V kappa regions A2 (SEQ ID NO: 373), A3 (SEQ ID NO: 371), A20 (SEQ ID NO: 370), A27 (SEQ ID NO: 369), A30 (SEQ ID NO: 374), L2 (SEQ ID NO: 372) or O1 (SEQ ID NO: 368); and the J region JK1 (SEQ ID NO: 389), JK2 (SEQ ID NO: 390), JK3 (SEQ ID NO: 391), JK4 (SEQ ID NO: 392) or JK5 (SEQ ID NO:
 30 393). (generally, see *Kabat Sequences of Proteins of Immunological Interest*, National Institutes of Health, Bethesda, Md. 1987 and 1991; also see Chothia & Lesk 1987 *J. Mol. Biol.* 196:901-917; Chothia et al. 1989 *Nature* 342:878-883). In a particular embodiment

of the invention human antibodies with binding specificity to GPNMB are combined germline regions as shown in Table 1.

TABLE 1: Human anti-GPNMB antibody germline region combinations.

Ab	VH	D	JH	VL	JL
1.10.2	VH4-59	D6-19	JH4b	A3	JK5
1.15.1	VH4-31	D1-20	JH4b	L2	JK1
1.2.2	VH2-5	D3-16	JH4b	O1	JK5
1.7.1	VH4-31	D1-20	JH4b	L2	JK1
2.10.2	VH3-30	D3-10	JH6b	A3	JK5
2.15.1	VH3-33	D4-17	JH4b	A20	JK4
2.16.1	VH3-11	D6-13	JH3b	L2	JK3
2.17.1	VH1-2	D6-19	JH5b	A2	JK4
2.21.2	VH3-21	D1-26	JH4b	A20	JK5
2.22.1	VH4-31	D3-22	JH6b	A30	JK1
2.24.1	VH5-51	D5-24	JH4b	A27	JK1
2.3.1	VH1-2	D3-10	JH4b	A2	JK4
2.7.1	VH3-33	D3-10	JH4b	A20	JK4
2.8.1	VH2-5	D3-9	JH4b	O1	JK4

- 5 In an embodiment of the invention, the isolated antibody has a heavy chain variable region polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 20, 38, 56, 74, 92, 110, 128, 146, 164, 182, 200, 218, 236, 253, 256, 260, 265, 270, 274, 277, 281 and 285. Such amino acid sequences can be encoded by nucleotide sequences selected from the group consisting of SEQ ID NOs: 1, 19, 37, 55, 73,
- 10 91, 109, 127, 145, 163, 181, 199, 217 and 235. In another embodiment, the invention provides an isolated antibody that specifically binds to GPNMB and has a light chain variable region polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 11, 29, 47, 65, 83, 101, 119, 137, 155, 173, 191, 209, 227 and 245. Such amino acid sequences can be encoded by nucleotide sequences selected from the
- 15 group consisting of SEQ ID NOs: 10, 28, 46, 64, 82, 100, 118, 136, 154, 172, 190, 208, 226 and 244. In yet another embodiment, the invention provides an isolated antibody that specifically binds to GPNMB and has a heavy chain polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 20, 38, 56, 74, 92, 110,

128, 146, 164, 182, 200, 218, 236, 253, 256, 260, 265, 270, 274, 277, 281 and 285 and has a light chain polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 11, 29, 47, 65, 83, 101, 119, 137, 155, 173, 191, 209, 227 and 245. In yet another embodiment of the invention, anti-GPNMB antibodies comprise at least one CDR of any of the H or L CDR polypeptide sequences SEQ ID NOs: 4, 6, 8, 13, 15, 17, 22, 24, 26, 31, 33, 35, 40, 42, 44, 49, 51, 53, 58, 60, 62, 67, 69, 71, 76, 78, 80, 85, 87, 89, 94, 96, 98, 103, 105, 107, 112, 114, 116, 121, 123, 125, 130, 132, 134, 139, 141, 143, 148, 150, 152, 157, 159, 161, 166, 168, 170, 175, 177, 179, 184, 186, 188, 193, 195, 197, 202, 204, 206, 211, 213, 215, 220, 222, 224, 229, 231, 233, 238, 240, 242, 247, 249, 251, 254, 257, 261, 266, 271, 278, 282, 286, 255, 258, 262, 267, 272, 275, 279, 283, 287, 259, 263, 264, 268, 269, 273, 276, 280, 284 and 288.

In particular embodiments, human anti-GPNMB antibodies are Mab1.10.2, Mab1.15.1, Mab1.2.2, Mab1.7.1, Mab2.10.2, Mab2.15.1, Mab2.16.1, Mab2.17.1, Mab2.21.2, Mab2.22.1, Mab2.24.1, Mab2.3.1, Mab2.7.1, and Mab2.8.1. These antibodies have amino acid sequences and nucleic acid sequences encoding them identified in this application as shown in Tables 2A-2D.

TABLE 2A Antibody Nucleotide (DNA) and Amino Acid (AA) Sequences

Gene Segment	1.10.2	1.15.1	1.2.2	1.7.1
H variable DNA	SEQ ID NO:1	SEQ ID NO:19	SEQ ID NO:37	SEQ ID NO:55
H variable AA	SEQ ID NO:2	SEQ ID NO:20	SEQ ID NO:38	SEQ ID NO:56
H FR1	SEQ ID NO:3	SEQ ID NO:21	SEQ ID NO:39	SEQ ID NO:57
H CDR1	SEQ ID NO:4	SEQ ID NO:22	SEQ ID NO:40	SEQ ID NO:58
H FR2	SEQ ID NO:5	SEQ ID NO:23	SEQ ID NO:41	SEQ ID NO:59
H CDR2	SEQ ID NO:6	SEQ ID NO:24	SEQ ID NO:42	SEQ ID NO:60
H FR3	SEQ ID NO:7	SEQ ID NO:25	SEQ ID NO:43	SEQ ID NO:61
H CDR3	SEQ ID NO:8	SEQ ID NO:26	SEQ ID NO:44	SEQ ID NO:62
H FR4	SEQ ID NO:9	SEQ ID NO:27	SEQ ID NO:45	SEQ ID NO:63
L variable DNA	SEQ ID NO:10	SEQ ID NO:28	SEQ ID NO:46	SEQ ID NO:64
L variable AA	SEQ ID NO:11	SEQ ID NO:29	SEQ ID NO:47	SEQ ID NO:65
L FR1	SEQ ID NO:12	SEQ ID NO:30	SEQ ID NO:48	SEQ ID NO:66
L CDR1	SEQ ID NO:13	SEQ ID NO:31	SEQ ID NO:49	SEQ ID NO:67
L FR2	SEQ ID NO:14	SEQ ID NO:32	SEQ ID NO:50	SEQ ID NO:68
L CDR2	SEQ ID NO:15	SEQ ID NO:33	SEQ ID NO:51	SEQ ID NO:69
L FR3	SEQ ID NO:16	SEQ ID NO:34	SEQ ID NO:52	SEQ ID NO:70
L CDR3	SEQ ID NO: 17	SEQ ID NO: 35	SEQ ID NO: 53	SEQ ID NO: 71
L FR4	SEQ ID NO:18	SEQ ID NO:36	SEQ ID NO:54	SEQ ID NO:72

TABLE 2B: Antibody Nucleotide (DNA) and Amino Acid (AA) Sequences

Gene Segment	2.10.2	2.15.1	2.16.1	2.17.1
H variable DNA	SEQ ID NO:73	SEQ ID NO:91	SEQ ID NO:109	SEQ ID NO:127
H variable AA	SEQ ID NO:74	SEQ ID NO:92	SEQ ID NO:110	SEQ ID NO:128
H FR1	SEQ ID NO:75	SEQ ID NO:93	SEQ ID NO:111	SEQ ID NO:129
H CDR1	SEQ ID NO:76	SEQ ID NO:94	SEQ ID NO:112	SEQ ID NO:130
H FR2	SEQ ID NO:77	SEQ ID NO:95	SEQ ID NO:113	SEQ ID NO:131
H CDR2	SEQ ID NO:78	SEQ ID NO:96	SEQ ID NO:114	SEQ ID NO:132
H FR3	SEQ ID NO:79	SEQ ID NO:97	SEQ ID NO:115	SEQ ID NO:133
H CDR3	SEQ ID NO:80	SEQ ID NO:98	SEQ ID NO:116	SEQ ID NO:134
H FR4	SEQ ID NO:81	SEQ ID NO:99	SEQ ID NO:117	SEQ ID NO:135
L variable DNA	SEQ ID NO:82	SEQ ID NO:100	SEQ ID NO:118	SEQ ID NO:136
L variable AA	SEQ ID NO:83	SEQ ID NO:101	SEQ ID NO:119	SEQ ID NO:137
L FR1	SEQ ID NO:84	SEQ ID NO:102	SEQ ID NO:120	SEQ ID NO:138
L CDR1	SEQ ID NO:85	SEQ ID NO:103	SEQ ID NO:121	SEQ ID NO:139
L FR2	SEQ ID NO:86	SEQ ID NO:104	SEQ ID NO:122	SEQ ID NO:140
L CDR2	SEQ ID NO:87	SEQ ID NO:105	SEQ ID NO:123	SEQ ID NO:141
L FR3	SEQ ID NO:88	SEQ ID NO:106	SEQ ID NO:124	SEQ ID NO:142
L CDR3	SEQ ID NO:89	SEQ ID NO:107	SEQ ID NO:125	SEQ ID NO:143
L FR4	SEQ ID NO:90	SEQ ID NO:108	SEQ ID NO:126	SEQ ID NO:144

TABLE 2C: Antibody Nucleotide (DNA) and Amino Acid (AA) Sequences

Gene Segment	2.21.2	2.22.1	2.24.1	2.3.1
H variable DNA	SEQ ID NO:145	SEQ ID NO:163	SEQ ID NO:181	SEQ ID NO:199
H variable AA	SEQ ID NO:146	SEQ ID NO:164	SEQ ID NO:182	SEQ ID NO:200
H FR1	SEQ ID NO:147	SEQ ID NO:165	SEQ ID NO:183	SEQ ID NO:201
H CDR1	SEQ ID NO:148	SEQ ID NO:166	SEQ ID NO:184	SEQ ID NO:202
H FR2	SEQ ID NO:149	SEQ ID NO:167	SEQ ID NO:185	SEQ ID NO:203
H CDR2	SEQ ID NO:150	SEQ ID NO:168	SEQ ID NO:186	SEQ ID NO:204
H FR3	SEQ ID NO:151	SEQ ID NO:169	SEQ ID NO:187	SEQ ID NO:205
H CDR3	SEQ ID NO:152	SEQ ID NO:170	SEQ ID NO:188	SEQ ID NO:206
H FR4	SEQ ID NO:153	SEQ ID NO:171	SEQ ID NO:189	SEQ ID NO:207
L variable DNA	SEQ ID NO:154	SEQ ID NO:172	SEQ ID NO:190	SEQ ID NO:208
L variable AA	SEQ ID NO:155	SEQ ID NO:173	SEQ ID NO:191	SEQ ID NO:209
L FR1	SEQ ID NO:156	SEQ ID NO:174	SEQ ID NO:192	SEQ ID NO:210
L CDR1	SEQ ID NO:157	SEQ ID NO:175	SEQ ID NO:193	SEQ ID NO:211
L FR2	SEQ ID NO:158	SEQ ID NO:176	SEQ ID NO:194	SEQ ID NO:212
L CDR2	SEQ ID NO:159	SEQ ID NO:177	SEQ ID NO:195	SEQ ID NO:213
L FR3	SEQ ID NO:160	SEQ ID NO:178	SEQ ID NO:196	SEQ ID NO:214
L CDR3	SEQ ID NO:161	SEQ ID NO:179	SEQ ID NO:197	SEQ ID NO:215
L FR4	SEQ ID NO:162	SEQ ID NO:180	SEQ ID NO:198	SEQ ID NO:216

TABLE 2D: Antibody Nucleotide (DNA) and Amino Acid (AA) Sequences

Gene Segment	2.7.1	2.8.1
H variable DNA	SEQ ID NO:217	SEQ ID NO:235
H variable AA	SEQ ID NO:218	SEQ ID NO:236
H FR1	SEQ ID NO:219	SEQ ID NO:237
H CDR1	SEQ ID NO:220	SEQ ID NO:238
H FR2	SEQ ID NO:221	SEQ ID NO:239
H CDR2	SEQ ID NO:222	SEQ ID NO:240
H FR3	SEQ ID NO:223	SEQ ID NO:241
H CDR3	SEQ ID NO:224	SEQ ID NO:242
H FR4	SEQ ID NO:225	SEQ ID NO:243
L variable DNA	SEQ ID NO:226	SEQ ID NO:244
L variable AA	SEQ ID NO:227	SEQ ID NO:245
L FR1	SEQ ID NO:228	SEQ ID NO:246
L CDR1	SEQ ID NO:229	SEQ ID NO:247
L FR2	SEQ ID NO:230	SEQ ID NO:248
L CDR2	SEQ ID NO:231	SEQ ID NO:249
L FR3	SEQ ID NO:232	SEQ ID NO:250
L CDR3	SEQ ID NO:233	SEQ ID NO:251
L FR4	SEQ ID NO:234	SEQ ID NO:252

VH4-31 derived anti-GPNMB Antibodies:

5 In a particular embodiment, GPNMB-binding human antibodies of the invention comprise germline V heavy chain region VH4-31 or are derived therefrom and have an amino acid sequence of the formula:

X₁SGPGLVKPSQX₂LSLTCTVS GGSIS SX₃X₄YX₅WX₆ WIRX₇HPGKGLEWIG
 10 YIYYSGX₈TYX₉NPSLKS RVX₁₀ISVDTSKNQFSLX₁₁LSSVTAADTAVYYCAR

Where: X₁ is E or Q;
 X₂ is T or N;
 X₃ is A, F or G;
 X₄ is N or G;
 X₅ is Y or F;
 15 X₆ is T or S;
 X₇ is Q or H;
 X₈ is S or N;
 X₉ is C, S or Y;
 X₁₀ is I or T;
 20 X₁₁ is K or T;
 (SEQ ID NO:253).

In specific embodiments SEQ ID NO:253 is combined with D3-22 or D1-20.

Furthermore the combination of SEQ ID NO:253 with D3-22 or D1-20 is combined with
 25 JH6b or JH4b and in specific embodiments, after affinity maturation these GPNMB-binding human antibodies, for example Mab1.15.1, Mab1.7.1 and Mab2.22.1, have amino acid sequences SEQ ID NOs:20, 56 and 164 and can be encoded by nucleotide sequences SEQ ID NO:19, 55 and 163.

Furthermore, in particular embodiments H chain CDR1 sequences are the germline VH4-31 CDR or affinity matured sequences thereof, of the formula:

CDR1: GGSIS SX₃X₄YX₅WX₆

Where: X3 is A, F or G;
X4 is N or G;
X5 is Y or F;
X6 is T or S;
(SEQ ID NO:254).

In specific embodiments an anti-GPNMB antibody of the invention comprise a CDR1 sequence selected from the following: SEQ ID NO:22, 58, 166.

In particular embodiments H chain CDR2 sequences are the germline VH4-31 CDR or affinity matured sequences thereof of the formula:

CDR2: YIYYSGX₈TYX₉NPSLKS

Where: X8 is S or N;
X9 is C, S or Y;
(SEQ ID NO:255).

In specific embodiments an anti-GPNMB antibody of the invention comprise a CDR2 sequence selected from the following: SEQ ID NO: 24, 60, and 168.

In particular embodiments, the H chain CDR3 sequence is a D3-22, JH6b combination having SEQ ID NO:170. Alternatively, in particular embodiments the H chain CDR3 sequence is a D1-20, JH4b combination having SEQ ID NO:26 or 62.

VH1-2 derived anti-GPNMB Antibodies:

In a particular embodiment, GPNMB-binding human antibodies of the invention comprise germline V heavy chain region VH1-2 or are derived therefrom and include an amino acid sequence of the formula:

QLVQSGAEVKKPGASVKVSCKAS GYTFT GX₁YMH WVRQX₂PGQGLEWMG
WINPNSGGTX₃YX₄QKFQX₅ RVTMTRDTSISTX₆YMELSRLRSDDTAVYYCAR

Where: X1 is Y or F;
X2 is A or T;
X3 is N or Y;
X4 is A or V;
X5 is D or G;
X6 is A or V;
(SEQ ID NO: 256).

In specific embodiments SEQ ID NO:256 is combined with D3-10 or D6-19. Furthermore the combination of SEQ ID NO:256 with D3-10 or D6-19 is combined with JH4b or JH5b and in specific embodiments, after affinity maturation these GPNMB-binding human antibodies, for example Mab2.3.1 and Mab 2.17.1 have amino acid sequences: SEQ ID NO:128 and 200 and can be encoded by nucleotide sequences SEQ ID NO:127 and 199.

Furthermore, in particular embodiments H chain CDR1 sequences are the germline VH1-2 CDR or affinity matured sequences thereof, of the formula:

CDR1: GYTFTGX₁YMH
Where: X₁ is Y or F,
(SEQ ID NO:257)

In specific embodiments an anti-GPNMB antibody of the invention comprise a CDR1 sequence selected from SEQ ID NO: 130 and 202.

In particular embodiments H chain CDR2 sequences are the germline VH1-2 CDR or affinity matured sequences thereof of the formula:

CDR2: WINPNSGGTX₃YX₄QKFQX₅,
Where: X₃ is N or Y;
X₄ is A or V;
X₅ is D or G
(SEQ ID NO:258).

In specific embodiments an anti-GPNMB antibody of the invention comprise a CDR2 sequence selected from SEQ ID NO:132 and 204.

In particular embodiments H chain CDR3 sequences are germline D3-10, JH4b combinations or affinity matured sequences thereof, having the amino acid sequence of the formula:

CDR3: X₁X₂X₃GSGSX₄X₅,
Where: X₁ is Y or D;
X₂ is Y or F;
X₃ is Y or F;
X₄ is Y or L;
X₅ is Y or L
(SEQ ID NO:259).

In specific embodiments an anti-GPNMB antibody of the invention comprise a CDR3 sequence selected from SEQ ID NO:134 and 206.

VH2-5 derived anti-GPNMB Antibodies:

In a particular embodiment, GPNMB-binding human antibodies of the invention comprise germline V heavy chain region VH2-5 or are derived therefrom and include an amino acid sequence of the formula:

ITLKESGPTLVX₁PTQTLTLTCTFS GFSLS X₂X₃GX₄GVG WIRQPPGKALX₅WLX₆
LIYWNDDKX₇YSPSLX₈S RLTITKDTSKNQVVLX₉X₁₀ TNMDPVDATYYCAH
Where: X₁ is K or T;
X₂ is T or A;
X₃ is S or G;
X₄ is M or V;
X₅ is D or E;
X₆ is A or T;
X₇ is R or H;
X₈ is K or R;
X₉ is T or R;

X10 is M or I;
(SEQ ID NO:260).

In specific embodiments SEQ ID NO:260 is combined with D3-9 or D3-16 and
5 furthermore is combined with JH4b. In specific embodiments, after affinity maturation
these GPNMB-binding human antibodies, for example, Mab 2.8.1 and Mab 1.2.2 have
amino acid sequences SEQ ID NO: 38 and 236 and can be encoded by nucleotide sequences
SEQ ID NO: 37 and 235.

Furthermore, in particular embodiments H chain CDR1 sequences are the germline
10 VH2-5 CDR or affinity matured sequences thereof, of the formula:

CDR1: GFSLS X₂X₃GX₄GVG
Where: X₂ is T or A;
X₃ is S or G;
X₄ is M or V;
15 (SEQ ID NO:261).

In specific embodiments an anti-GPNMB antibody of the invention comprise a
CDR1 sequence selected from SEQ ID NO: 40 and 238.

In particular embodiments H chain CDR2 sequences are the germline VH2-5 CDR2
20 or affinity matured sequences thereof of the formula:

CDR2: LIYWNDCKX₇YSPSLX₈S
Where: X₇ is R or H;
X₈ is K or R;
25 (SEQ ID NO:262).

In specific embodiments an anti-GPNMB antibody of the invention comprise a
CDR2 sequence selected from SEQ ID NO:42 and 240.

In particular embodiments H chain CDR3 sequences are germline D3-9, JH4b
combinations or affinity matured sequences thereof and include an amino acid sequence of
30 the formula:

CDR3: X₁YDILTGX₂X₃
Where: X₁ is Y or H;
X₂ is Y or F; and
X₃ is Y or N
35 (SEQ ID NO:263).

In a specific embodiments an anti-GPNMB antibody of the invention comprises a
CDR3 amino acid sequence SEQ ID NO:242.

In yet another particular embodiment H chain CDR3 sequences are germline D3-16,
40 JH4b combinations or affinity matured sequences thereof and include an amino acid
sequence of the formula:

CDR3:YDYX₁WGS

Where: X1 is V or D
(SEQ ID NO:264).

In a specific embodiment an anti-GPNMB antibody of the invention comprises a
5 CDR3 amino acid sequence SEQ ID NO: 44.

VH3-33 derived anti-GPNMB Antibodies:

In a particular embodiment, GPNMB-binding human antibodies of the invention
comprise germline V heavy chain region VH3-33 or are derived therefrom and have an
10 amino acid sequence of the formula:

QVQLX₁X₂SGGGVVQPGRSLRLSCAAS GFTFX₃X₄YGX₅H WVRQAPGKGLEWVA
VIWX₆DGX₇NKYYADSVKG RFTISRDNKNTLYLQMNSLRAEDX₈AVYYCAX₉

Where: X1 is V or E;

X2 is E or Q;

15 X3 is S or N;

X4 is S or N;

X5 is M or I;

X6 is Y or F;

X7 is S or R;

20 X8 is T or A;

X9 is R or K

(SEQ ID NO:265).

In specific embodiments SEQ ID NO:265 is combined with D3-10 or D4-17 and
25 furthermore with JH4b. In specific embodiments, after affinity maturation these GPNMB-
binding human antibodies, for example Mab 2.7.1 and Mab2.15.1 have amino acid
sequences: SEQ ID NO:92 and 218 and can be encoded by nucleotide sequences SEQ ID
NO:91 and 217.

Furthermore, in particular embodiments H chain CDR1 sequences are the germline
30 VH3-33 CDR or affinity matured sequences thereof, of the formula:

CDR1: GFTFX₃X₄YGX₅H

Where: X3 is S or N;

X4 is S or N;

35 X5 is M or I;

(SEQ ID NO:266).

In specific embodiments an anti-GPNMB antibody of the invention comprise a
CDR1 amino acid sequence selected from SEQ ID NO:94 and 220.

In particular embodiments H chain CDR2 sequences are the germline VH3-33
40 CDR2 or affinity matured sequences thereof of the formula:

CDR2: VIWX₆DGX₇NKYYADSVKG

Where: X6 is Y or F;

X7 is S or R;

45 (SEQ ID NO:267).

In specific embodiments an anti-GPNMB antibody of the invention comprise a CDR2 sequence selected from SEQ ID NO:96 and 222.

In particular embodiments H chain CDR3 sequences are D3-10, JH4b combinations or affinity matured sequences thereof and include an amino acid sequence of the formula:

5 CDR3: YYYGSGX₁
Where: X₁ is S or L
(SEQ ID NO:268).

A specific embodiment is anti-GPNMB antibody 2.7.1 having a CDR3 amino acid sequence SEQ ID NO:224.

In an alternative embodiment H chain CDR3 sequences are D4-17, JH4b combinations or affinity matured sequences thereof and include an amino acid sequence of the formula:

15 CDR3: DYGD₁X₁
Where: X₁ is Y or S
(SEQ ID NO:269).

A specific embodiment is anti-GPNMB antibody 2.15.1 having a CDR3 amino acid sequence SEQ ID NO: 98.

VH3-11 derived anti-GPNMB Antibodies:

20 In a particular embodiment, GPNMB-binding human antibodies of the invention comprise germline V heavy chain region VH3-11 or are derived therefrom and have an amino acid sequence of the formula:

25 QVQLVESGGGLVKPGGSLRLS₁CAAS GFTFS X₁YX₂MX₃ WIRQAPGKGLEWVS
YISX₄SGSX₅X₆X₇YADSVKG RFTX₈SRDNAKNSLYLQMNSLRAEDTAVYYCAR
Where: X₁ is D or S;
X₂ is S or Y;
X₃ is S or T;
X₄ is S or I;
30 X₅ is T or I;
X₆ is T or I;
X₇ is Y or H;
X₈ is I or M;
(SEQ ID NO:270).

35 In specific embodiments SEQ ID NO:270 is combined with D6-13 and furthermore with JH3b. In specific embodiments, after affinity maturation these GPNMB-binding human antibodies, for example Mab 2.16.1 have amino acid sequence SEQ ID NO:110 and can be encoded by nucleotide sequence SEQ ID NO:109.

40 Furthermore, in particular embodiments H chain CDR1 sequences are the germline VH3-11 CDR1 or affinity matured sequences thereof, of the formula:

CDR1: GFTFS X₁YX₂MX₃
Where: X₁ is D or S;

X2 is S or Y;
 X3 is S or T;
 (SEQ ID NO:271).

5 In specific embodiments an anti-GPNMB antibody of the invention comprise a CDR1 amino acid sequence SEQ ID NO:112.

In particular embodiments H chain CDR2 sequences are the germline VH3-11 CDR2 or affinity matured sequences thereof of the formula:

10 CDR2: YISX₄SGSX₅X₆X₇YADSVKG
 Where: X4 is S or I;
 X5 is T or I;
 X6 is T or I;
 X7 is Y or H;
 (SEQ ID NO:272).

15 In specific embodiments an anti-GPNMB antibody of the invention comprises a CDR2 sequence SEQ ID NO:114.

In particular embodiments H chain CDR3 sequences are D6-13, JH3b combinations or affinity matured sequences thereof and include an amino acid sequence of the formula:

20 CDR3: X₁X₂AAAG- - AFDI
 Where: X1 is G or D;
 X2 is I or G;
 (SEQ ID NO:273).

25 A specific embodiment is anti-GPNMB antibody 2.16.1 having a CDR3 amino acid sequence SEQ ID NO:116.

VH3-21 derived anti-GPNMB Antibodies:

30 In a particular embodiment, GPNMB-binding human antibodies of the invention comprise germline V heavy chain region VH3-21 or are derived therefrom and have an amino acid sequence of the formula:

35 X₁VQLX₂X₃SGGGLVKPGGSLRX₄SCAASGFTFSYSMNWVRQAPGKGGLEWVSX₅ISS
 SSSYIYYADSVKG RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR
 Where: X1 is E or Q;
 X2 is V or E;
 X3 is E or Q;
 X4 is F or L;
 X5 is S or F;
 (SEQ ID NO:274).

40 In specific embodiments SEQ ID NO:274 is combined with D1-26 and furthermore with JH4b. In specific embodiments, after affinity maturation these GPNMB-binding human antibodies, for example Mab 2.21.1 have amino acid sequence SEQ ID NO:146 and can be encoded by nucleotide sequence SEQ ID NO:145.

Furthermore, in particular embodiments H chain CDR1 sequences are the germline

VH3-21 CDR1, SEQ ID NO:148 or affinity matured sequences thereof.

In particular embodiments H chain CDR2 sequences are the germline VH3-21 CDR2 or affinity matured sequences thereof of the formula:

CDR2: X₅ISS SSSYIYYADSVKG

Where: X₅ is S or F;

(SEQ ID NO:275).

In specific embodiments an anti-GPNMB antibody of the invention comprises a CDR2 amino acid sequence SEQ ID NO:150.

In particular embodiments H chain CDR3 sequences are D1-26, JH4b combinations or affinity matured sequences thereof and include an amino acid sequence of the formula:

CDR3: X₁X₂VGAT-FDY

Where: X₁ is G or D;

X₂ is I or W;

(SEQ ID NO:276).

A specific embodiment is anti-GPNMB antibody 2.21.1 having a CDR3 amino acid sequence SEQ ID NO:152.

VH3-30 derived anti-GPNMB Antibodies:

In a particular embodiment, GPNMB-binding human antibodies of the invention comprise germline V heavy chain region VH3-30 or are derived therefrom and include an amino acid sequence of the formula:

QLVESGGGVVQPGRSLRLSCAAS GFX₁FS SYGMH WVRQAPGKGLEWVA
VISYDGX₂NKYIYADSVKG RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAK

Where: X₁ is T or A;

X₂ is S or N;

(SEQ ID NO:277).

In specific embodiments SEQ ID NO:277 is combined with D3-10 and furthermore with JH6b. In specific embodiments, after affinity maturation these GPNMB-binding human antibodies, for example Mab 2.10.2 have amino acid sequence SEQ ID NO:74 and can be encoded by nucleotide sequence SEQ ID NO:73.

Furthermore, in particular embodiments H chain CDR1 sequences are the germline VH3-30 CDR1, or affinity matured sequences thereof having an amino acid sequence of the formula:

GFX₁FS SYGMH

Where: X₁ is T or A;

(SEQ ID NO:278).

In specific embodiments an anti-GPNMB antibody of the invention comprise a CDR1 sequence SEQ ID NO:76.

In particular embodiments H chain CDR2 sequences are the germline VH3-30 CDR2 or affinity matured sequences thereof of the formula:

CDR2: VISYD_{X2}GNKY_{X2}YADSVKG

Where: X2 is S or N;

(SEQ ID NO:279).

In specific embodiments an anti-GPNMB antibody of the invention comprises a

5 CDR2 amino acid sequence SEQ ID NO:78.

In particular embodiments H chain CDR3 sequences are D3-10, JH6b combinations or affinity matured sequences thereof and include an amino acid sequence of the formula:

CDR3: X₁X₂X₃VRGX₄X₅X₆

Where: X1 is I or D;

X2 is T or L;

X3 is M or V;

X4 is V or I;

X5 is I or R;

X6 is I or G;

(SEQ ID NO:280).

A specific embodiment is anti-GPNMB antibody 2.10.2 having a CDR3 amino acid sequence SEQ ID NO:80.

VH4-59 derived anti-GPNMB Antibodies:

20

In a particular embodiment, GPNMB-binding human antibodies of the invention comprise germline V heavy chain region VH4-59 or are derived therefrom and include an amino acid sequence of the formula:

QVQLQESGPGLVKPSETLSLTCTVS GX₁S₁S₂ X₂YYWS WIRQPPGKGLEWIG
YX₃YYSGSTNYPNPSLKS RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR

25

Where: X1 is G or D;

X2 is S or N;

X3 is I or F;

(SEQ ID NO:281).

30

In specific embodiments SEQ ID NO:281 is combined with D6-19 and furthermore with JH4b. In specific embodiments, after affinity maturation these GPNMB-binding human antibodies, for example Mab 1.10.2 have amino acid sequence SEQ ID NO:2 and can be encoded by nucleotide sequence SEQ ID NO:1.

35

Furthermore, in particular embodiments H chain CDR1 sequences are the germline VH4-59 CDR1, or affinity matured sequences thereof having an amino acid sequence of the formula:

GX₁S₁S₂ X₂YYWS

Where: X1 is G or D;

X2 is S or N;

(SEQ ID NO:282).

40

In specific embodiments an anti-GPNMB antibody of the invention comprise a CDR1 sequence SEQ ID NO:4.

In particular embodiments H chain CDR2 sequences are the germline VH4-59 CDR2 or affinity matured sequences thereof of the formula:

CDR2: YX₃YYSGSTNYNPSLKS

Where: X₃ is I or F;

(SEQ ID NO:283).

In specific embodiments an anti-GPNMB antibody of the invention comprises a CDR2 amino acid sequence SEQ ID NO:6.

In particular embodiments H chain CDR3 sequences are D6-19, JH4b combinations or affinity matured sequences thereof and include an amino acid sequence of the formula:

CDR3: X₁X₂GW---DY

Where: X₁ is S or D;

X₂ is S or R;

(SEQ ID NO:284).

A specific embodiment is anti-GPNMB antibody 1.10.2 having a CDR3 amino acid sequence SEQ ID NO:8.

VH5-51 derived anti-GPNMB Antibodies:

In a particular embodiment, GPNMB-binding human antibodies of the invention comprise germline V heavy chain region VH5-51 or are derived therefrom and include an amino acid sequence of the formula:

QLVQSGAEVKKPGESLKISCX₁GS GYX₂FT X₃YWIG WVRQMPQKGLEWMG
X₄LYPX₅DSDTRYSPSFQG QVTISADKSISTAYLQWSSLKASDTAX₆YYCAR

Where: X₁ is K or Q;

X₂ is S or I;

X₃ is S or N;

X₄ is I or V;

X₅ is G or D;

X₆ is M or I;

(SEQ ID NO:285).

In specific embodiments SEQ ID NO:285 is combined with D5-24 and furthermore with JH4b. In specific embodiments, after affinity maturation these GPNMB-binding human antibodies, for example Mab 2.24.1 have amino acid sequence SEQ ID NO:182 and can be encoded by nucleotide sequence SEQ ID NO:181.

Furthermore, in particular embodiments H chain CDR1 sequences are the germline VH5-51 CDR1, or affinity matured sequences thereof having an amino acid sequence of the formula:

GYX₂FT X₃YWIG

Where: X₂ is S or I;

X₃ is S or N;

(SEQ ID NO:286).

In specific embodiments an anti-GPNMB antibody of the invention comprise a CDR1 sequence SEQ ID NO:184.

In particular embodiments H chain CDR2 sequences are the germline VH5-51 CDR2 or affinity matured sequences thereof of the formula:

5 CDR2: X₄IYPX₅DSDTRYSPSFQG
Where: X₄ is I or V;
X₅ is G or D;
(SEQ ID NO:287).

In specific embodiments an anti-GPNMB antibody of the invention comprises a CDR2 amino acid sequence SEQ ID NO:186.

In particular embodiments H chain CDR3 sequences are D5-24, JH4b combinations or affinity matured sequences thereof and include an amino acid sequence of the formula:

15 CDR3: X₁WLQX₂--FDY
Where: X₁ is R or K;
X₂ is L or H;
(SEQ ID NO:288).

A specific embodiment is anti-GPNMB antibody 2.24.1 having a CDR3 amino acid sequence SEQ ID NO:188.

20 The antibodies of the invention bind an epitope of GPNMB (SEQ ID NO:289), preferably within the mature sequence of GPNMB and more preferably within the extracellular domain (ECD) of GPNMB.

Antibodies of the invention bind GPNMB with an affinity of 10⁻⁶ to 10⁻¹¹. Preferably with an affinity of 10⁻⁷ or greater and even more preferably 10⁻⁸ or greater. In a preferred embodiment, antibodies described herein bind to GPNMB with very high affinities (K_d), for example a human antibody that is capable of binding GPNMB with a K_d less than, but not limited to, 10⁻⁷, 10⁻⁸, 10⁻⁹, 10⁻¹⁰, 10⁻¹¹, 10⁻¹², 10⁻¹³ or 10⁻¹⁴ M, or any range or value therein. Affinity and/or avidity measurements can be measured by KinExA[®] and/or BIACORE[®], as described herein. In particular embodiments antibodies of the invention bind to GPNMB with K_ds ranging from 50 to 150 pM.

30 Epitope mapping and secondary and tertiary structure analyses can be carried out to identify specific 3D structures assumed by the disclosed antibodies and their complexes with antigens (see, e.g., *Epitope Mapping Protocols*, ed. Morris, Humana Press, 1996). Such methods include, but are not limited to, X-ray crystallography (*Biochem. Exp. Biol.*, 11:7-13, 1974) and computer modeling of virtual representations of the presently disclosed antibodies (Fletterick et al. (1986) *Computer Graphics and Molecular Modeling, in Current*

Communications in Molecular Biology, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).

Furthermore, the specific part of the protein immunogen recognized by antibody may be determined by assaying the antibody reactivity to parts of the protein, for example an N terminal and C terminal half. The resulting reactive fragment can then be further dissected, assaying consecutively smaller parts of the immunogen with the antibody until the minimal reactive peptide is defined. Alternatively, the binding specificity, that is the epitope, of anti-GPNMB antibodies of the invention may be determined by subjecting GPNMB immunogen to SDS-PAGE either in the absence or presence of a reduction agent and analyzed by immunoblotting. Epitope mapping may also be performed using SELDI. SELDI ProteinChip® (LumiCyte) arrays used to define sites of protein-protein interaction. GPNMB protein antigen or fragments thereof may be specifically captured by antibodies covalently immobilized onto the PROTEINCHIP array surface. The bound antigens may be detected by a laser-induced desorption process and analyzed directly to determine their mass.

The epitope recognized by anti-GPNMB antibodies described herein may be determined by exposing the PROTEINCHIP Array to a combinatorial library of random peptide 12-mer displayed on Filamentous phage (New England Biolabs). Antibody-bound phage are eluted and then amplified and taken through additional binding and amplification cycles to enrich the pool in favor of binding sequences. After three or four rounds, individual binding clones are further tested for binding by phage ELISA assays performed on antibody-coated wells and characterized by specific DNA sequencing of positive clones.

Derivatives

This disclosure also provides a method for obtaining an antibody specific for GPNMB. CDRs in such antibodies are not limited to the specific sequences of H and L variable domains identified in Table 1 and may include variants of these sequences that retain the ability to specifically bind GPNMB. Such variants may be derived from the sequences listed in Table 1 by a skilled artisan using techniques well known in the art. For example, amino acid substitutions, deletions, or additions, can be made in the FRs and/or in the CDRs. While changes in the FRs are usually designed to improve stability and immunogenicity of the antibody, changes in the CDRs are typically designed to increase affinity of the antibody for its target. Variants of FRs also include naturally occurring immunoglobulin allotypes. Such affinity-increasing changes may be determined empirically

by routine techniques that involve altering the CDR and testing the affinity of the antibody for its target. For example, conservative amino acid substitutions can be made within any one of the disclosed CDRs. Various alterations can be made according to the methods described in the art (*Antibody Engineering*, 2^{sup.nd} ed., Oxford University Press, ed. Borrebaeck, 1995). These include but are not limited to nucleotide sequences that are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a "silent" change. For example, the nonpolar amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine, and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs (see Table 3). Furthermore, any native residue in the polypeptide may also be substituted with alanine (*Acta Physiol. Scand. Suppl.* 643:55-67, 1998; *Adv. Biophys.* 35:1-24, 1998).

TABLE 3. Amino acid substitutions

Original aa Residue	Possible Substitutions	Prefered substitution
Ala (A)	Val, Leu, Ile	Val
Arg (R)	Lys, Gln, Asn	Lys
Asn (N)	Gln	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser, Ala	Ser
Gln (Q)	Asn	Asn
Gly (G)	Pro, Ala	Ala
His (H)	Asn, Gln, Lys, Arg	Arg
Ile (I)	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu (L)	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys (K)	Arg, 1,4-Diamino-butyric Acid, Gln, Asn	Arg
Met (M)	Leu, Phe, Ile	Leu
Phe (F)	Leu, Val, Ile, Ala, Tyr	Leu
Pro (P)	Ala Gly	Gly
Ser (S)	Thr, Ala, Cys	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr, Phe	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser	Phe
Val (V)	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

Derivatives and analogs of antibodies of the invention can be produced by various techniques well known in the art, including recombinant and synthetic methods (*Maniatis* (1990) *Molecular Cloning, A Laboratory Manual*, 2^{sup.nd} ed., Cold Spring Harbor

Laboratory, Cold Spring Harbor, N.Y., and Bodansky et al. (1995) *The Practice of Peptide Synthesis*, 2nd ed., Springer Verlag, Berlin, Germany).

Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and (4) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various mutants of a sequence other than the naturally-occurring peptide sequence. For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the naturally-occurring sequence (preferably in the portion of the polypeptide outside the domain(s) forming intermolecular contacts). A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in the art (for example, *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman and Company, New York (1984)).

In one embodiment, a method for making an H variable domain which is an amino acid sequence variant of an H variable domain of the invention comprises a step of adding, deleting, substituting, or inserting one or more amino acids in the amino acid sequence of the presently disclosed H variable domain, optionally combining the H variable domain thus provided with one or more L variable domains, and testing the H variable domain or H variable/L variable combination or combinations for specific binding to GPNMB or and, optionally, testing the ability of such antigen-binding domain to modulate GPNMB activity. The L variable domain may have an amino acid sequence that is identical or is substantially as set out according to Table 1.

An analogous method can be employed in which one or more sequence variants of a L variable domain disclosed herein are combined with one or more H variable domains.

A further aspect of the disclosure provides a method of preparing antigen-binding fragment that specifically binds with GPNMB. The method comprises: (a) providing a starting repertoire of nucleic acids encoding a H variable domain that either includes a CDR3 to be replaced or lacks a CDR3 encoding region; (b) combining the repertoire with a donor nucleic acid encoding an amino acid sequence substantially as set out herein for a H variable CDR3 such that the donor nucleic acid is inserted into the CDR3 region in the

repertoire, so as to provide a product repertoire of nucleic acids encoding a H variable domain; (c) expressing the nucleic acids of the product repertoire; (d) selecting a binding fragment specific for GPNMB; and (e) recovering the specific binding fragment or nucleic acid encoding it.

5 Again, an analogous method may be employed in which a L variable CDR3 of the invention is combined with a repertoire of nucleic acids encoding a L variable domain, which either include a CDR3 to be replaced or lack a CDR3 encoding region. The donor nucleic acid may be selected from nucleic acids encoding an amino acid sequence substantially as set out in SEQ ID NOs: 2, 20, 38, 56, 74, 92, 110, 128, 146, 164, 182, 200,
10 218, 236, 253, 256, 260, 265, 270, 274, 277, 281, 285, 11, 29, 47, 65, 83, 101, 119, 137, 155, 173, 191, 209, 227 and 245. A sequence encoding a CDR of the invention (*e.g.*, CDR3) may be introduced into a repertoire of variable domains lacking the respective CDR (*e.g.*, CDR3), using recombinant DNA technology, for example, using methodology described by Marks *et al.* (*Bio/Technology* (1992) 10: 779-783). In particular, consensus primers directed
15 at or adjacent to the 5' end of the variable domain area can be used in conjunction with consensus primers to the third framework region of human H variable genes to provide a repertoire of H variable domains lacking a CDR3. The repertoire may be combined with a CDR3 of a particular antibody. Using analogous techniques, the CDR3-derived sequences may be shuffled with repertoires of H variable or L variable domains lacking a CDR3, and
20 the shuffled complete H variable or L variable domains combined with a cognate L variable or H variable domain to make the GPNMB specific antibodies of the invention. The repertoire may then be displayed in a suitable host system such as the phage display system such as described in WO92/01047 so that suitable antigen-binding fragments can be selected.

25 Analogous shuffling or combinatorial techniques may be used (*e.g.* Stemmer, *Nature* (1994) 370: 389-391). In further embodiments, one may generate novel H variable or L variable regions carrying one or more sequences derived from the sequences disclosed herein using random mutagenesis of one or more selected H variable and/or L variable genes, such as error-prone PCR (*Proc. Nat. Acad. Sci. U.S.A.* (1992) 89: 3576-3580).
30 Another method that may be used is to direct mutagenesis to CDRs of H variable or L variable genes (*Proc. Nat. Acad. Sci. U.S.A.* (1994) 91: 3809-3813; *J. Mol. Biol.* (1996) 263: 551-567). Similarly, one or more, or all three CDRs may be grafted into a repertoire of

H variable or L variable domains, which are then screened for an antigen-binding fragment specific for GPNMB.

A portion of an immunoglobulin variable domain will comprise at least one of the CDRs substantially as set out herein and, optionally, intervening framework regions as set out herein. The portion may include at least about 50% of either or both of FR1 and FR4, the 50% being the C-terminal 50% of FR1 and the N-terminal 50% of FR4. Additional residues at the N-terminal or C-terminal end of the substantial part of the variable domain may be those not normally associated with naturally occurring variable domain regions. For example, construction of antibodies by recombinant DNA techniques may result in the introduction of N- or C-terminal residues encoded by linkers introduced to facilitate cloning or other manipulation steps. Other manipulation steps include the introduction of linkers to join variable domains to further protein sequences including immunoglobulin heavy chain constant regions, other variable domains (for example, in the production of diabodies), or proteinaceous labels as discussed in further detail below.

Although the embodiments illustrated in the Examples comprise a "matching" pair of H variable and L variable domains, a skilled artisan will recognize that alternative embodiments may comprise antigen-binding fragments containing only a single CDR from either L variable or H variable domain. Either one of the single chain specific binding domains can be used to screen for complementary domains capable of forming a two-domain specific antigen-binding fragment capable of, for example, binding to GPNMB. The screening may be accomplished by phage display screening methods using the so-called hierarchical dual combinatorial approach disclosed in WO92/01047, in which an individual colony containing either an H or L chain clone is used to infect a complete library of clones encoding the other chain (L or H) and the resulting two-chain specific binding domain is selected in accordance with phage display techniques as described.

Anti-GPNMB antibodies described herein can be linked to another functional molecule, *e.g.*, another peptide or protein (albumin, another antibody, etc.), toxin, radioisotope, cytotoxic or cytostatic agents. For example, the antibodies can be linked by chemical cross-linking or by recombinant methods. The antibodies may also be linked to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192; or 4,179,337. The antibodies can be chemically modified by covalent conjugation to a polymer, for example, to increase their circulating half-life.

Exemplary polymers and methods to attach them are also shown in U.S. Pat. Nos. 4,766,106; 4,179,337; 4,495,285, and 4,609,546.

The disclosed antibodies may also be altered to have a glycosylation pattern that differs from the native pattern. For example, one or more carbohydrate moieties can be deleted and/or one or more glycosylation sites added to the original antibody. Addition of glycosylation sites to the presently disclosed antibodies may be accomplished by altering the amino acid sequence to contain glycosylation site consensus sequences known in the art. Another means of increasing the number of carbohydrate moieties on the antibodies is by chemical or enzymatic coupling of glycosides to the amino acid residues of the antibody (WO 87/05330; *CRC Crit. Rev. Biochem.*, 22: 259-306, 1981). Removal of any carbohydrate moieties from the antibodies may be accomplished chemically or enzymatically (*Arch. Biochem. Biophys.*, 259: 52, 1987; *Anal. Biochem.*, 118: 131, 1981; *Meth. Enzymol.*, 138: 350, 1987). The antibodies may also be tagged with a detectable, or functional, label. Detectable labels include radiolabels such as ^{131}I or ^{99}Tc , which may also be attached to antibodies using conventional chemistry. Detectable labels also include enzyme labels such as horseradish peroxidase or alkaline phosphatase. Detectable labels further include chemical moieties such as biotin, which may be detected via binding to a specific cognate detectable moiety, e.g., labeled avidin.

The valency of the antibodies may be custom designed to affect affinity and avidity, retention time at binding sites (see e.g. *Am H. Pathol*, 2002 160:1597-1608; *J. Med. Chem.* 2002 45:2250-2259; *Br. J. Cancer* 2002 86:1401-1410; *Biomol. Eng.* 2001 18:95-108; *Int J. Cancer* 2002 100:367-374).

Multiple specificity (bifunctional) binding reagents may be designed based upon the GPNMB specific sequences of the invention (*Biomol. Eng.* 2001 18:31-40). For example, a bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments (*Clin. Exp. Immunol.* 1990, 79: 315-321; *J. Immunol.* 199, 2148:1547-1553). Such bispecific antibodies can be generated comprising a specificity to GPNMB and a second specificity to a second molecule using techniques that are well known (*Immunol Methods* 1994, 4:72-81; *Wright and Harris, supra.*; *Trautnecker et al.* 1992 *Int. J. Cancer (Suppl.)* 7:51-52). Bispecific antibodies prepared in this manner selectively kill cells expressing GPNMB.

Antibodies, in which CDR sequences differ only insubstantially from those set out in SEQ ID NOs: 4, 6, 8, 13, 15, 17, 22, 24, 26, 31, 33, 35, 40, 42, 44, 49, 51, 53, 58, 60, 62, 67, 69, 71, 76, 78, 80, 85, 87, 89, 94, 96, 98, 103, 105, 107, 112, 114, 116, 121, 123, 125, 130, 132, 134, 139, 141, 143, 148, 150, 152, 157, 159, 161, 166, 168, 170, 175, 177, 179, 184, 186, 188, 193, 195, 197, 202, 204, 206, 211, 213, 215, 220, 222, 224, 229, 231, 233, 238, 240, 242, 247, 249 and 251. And formulas: 254, 257, 261, 266, 271, 278, 282, 286, 255, 258, 262, 267, 272, 275, 279, 283, 287, 259, 263, 264, 268, 269, 273, 276, 280, 284, 288, are encompassed within the scope of this invention. Typically, an amino acid is substituted by a related amino acid having similar charge, hydrophobic, or stereochemical characteristics. Such substitutions would be within the ordinary skills of an artisan. Unlike in CDRs, more substantial changes can be made in FRs without adversely affecting the binding properties of an antibody. Changes to FRs include, but are not limited to engineering certain framework residues that are important for antigen contact or for stabilizing the binding site, *e.g.*, changing the class or subclass of the constant region, changing specific amino acid residues which might alter the effector function such as Fc receptor binding (*U.S. Pat. Nos. 5,624,821; 5,648,260; Lund et al. (1991) J. Immun. 147: 2657-2662; Morgan et al. (1995) Immunology 86: 319-324*), or changing the species from which the constant region is derived.

One of skill in the art will appreciate that the derivatives and modifications described above are not all-exhaustive, and that many other modifications would be obvious to a skilled artisan in light of the teachings of the present disclosure.

Nucleic Acids, Cloning and Expression Systems

The present disclosure further provides isolated nucleic acids encoding the disclosed antibodies. The nucleic acids may comprise DNA or RNA and may be wholly or partially synthetic or recombinant. Reference to a nucleotide sequence as set out herein encompasses a DNA molecule with the specified sequence, and encompasses a RNA molecule with the specified sequence in which U is substituted for T, unless context requires otherwise.

The nucleic acids provided herein comprise a coding sequence for a CDR, a H variable domain, and/or a L variable domain disclosed herein.

The present disclosure also provides constructs in the form of plasmids, vectors, phagemids, transcription or expression cassettes which comprise at least one nucleic acid encoding a CDR, a H variable domain, and/or a L variable domain disclosed here.

The disclosure further provides a host cell which comprises one or more constructs as above.

Also provided are nucleic acids encoding any CDR (CDR1, CDR2, CDR3 from either the H or L variable domain), H variable or L variable domain, as well as methods of making of the encoded products. The method comprises expressing the encoded product from the encoding nucleic acid. Expression may be achieved by culturing under appropriate conditions recombinant host cells containing the nucleic acid. Following production by expression, a H variable or L variable domain, or specific binding member may be isolated and/or purified using any suitable technique, then used as appropriate.

Antigen-binding fragments, H variable and/or L variable domains and encoding nucleic acid molecules and vectors may be isolated and/or purified from their natural environment, in substantially pure or homogeneous form, or, in the case of nucleic acid, free or substantially free of nucleic acid or genes of origin other than the sequence encoding a polypeptide with the required function.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known in the art including cells suitable for producing antibodies (*Gene Expression Systems, Academic Press, eds. Fernandez et al., 1999*). Briefly, suitable host cells include bacteria, plant cells, mammalian cells, and yeast and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney cells, NS0 mouse myeloma cells, and many others. A common bacterial host is *E. coli*. Any protein expression system compatible with the invention may be used to produce the disclosed antibodies. Suitable expression systems also include transgenic animals (*Gene Expression Systems, Academic Press, eds. Fernandez et al., 1999*).

Suitable vectors can be chosen or constructed, so that they contain appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids or viral, e.g., phage, or phagemid, as appropriate (see *Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, 1989*). Many known techniques and protocols for manipulation of nucleic acid, for example, in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are known in the art (*Current*

Protocols in Molecular Biology, 2.sup.nd Edition, eds. Ausubel et al., John Wiley & Sons, 1992).

The invention also provides a host cell comprising a nucleic acid as disclosed herein. A still further aspect provides a method comprising introducing such nucleic acid into a host
5 cell. The introduction may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g., vaccinia or, for insect cells, baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage. The
10 introduction of the nucleic acid into the cells may be followed by causing or allowing expression from the nucleic acid, e.g., by culturing host cells under conditions for expression of the gene.

Immunoconjugates

In another aspect, the antibodies of the invention can be used as a targeting agent for
15 delivery of another therapeutic or a cytotoxic agent to a cell expressing GPNMB. The method includes administering an anti-GPNMB antibody coupled to a therapeutic or a cytotoxic agent or under conditions that allow binding of the antibody to GPNMB.

Anti-GPNMB antibodies are conjugated to a therapeutic agent, such as a cytotoxic compound, such that the resulting immunoconjugate exerts a cytotoxic or cytostatic effect
20 on a GPNMB expressing cell. Particularly suitable moieties for conjugation to antibodies are chemotherapeutic agents, prodrug converting enzymes or toxins. For example, an anti-GPNMB antibody can be conjugated to a cytotoxic agent such as a chemotherapeutic agent (see infra) or a toxin (e.g. abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin). Alternatively, anti-GPNMB antibody may be conjugated to a pro-drug converting enzyme.

25 The pro-drug converting enzyme can be recombinantly fused to the antibody or derivative thereof or chemically conjugated thereto using known methods. Exemplary pro-drug converting enzymes are carboxypeptidase G2, β -glucuronidase, penicillin-V-amidase, penicillin-G-amidase, β -lactamase, β -glucosidase, nitroreductase and carboxypeptidase A.

Any agent that exerts a therapeutic effect on GPNMB expressing cells can be used
30 as an agent for conjugation to an anti-GPNMB antibody of the invention. Useful classes of cytotoxic agents include, for example, antitubulin agents, auristatins, DNA minor groove binders, NDA replication inhibitors, alkylating agents (e.g., platinum complexes such as cis-platin, mono(platinum), bis(platinum) and tri-nuclear platinum complexes and

carboplatin), anthracyclines, antitumorics, antifolates, antimetabolites, chemotherapy sensitizers, duocarmycins, etoposides, fluorinated purimidines, ionophores, lexitropsins, nitrosoureas, platinols, pre-forming compounds, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, vinca alkaloids, or the like.

5 The therapeutic agent can be a cytotoxic agent. Suitable cytotoxic agents include, for example, dolastatins (*e.g.* auristatin E, AFP, MMAF, MMAE), DNA minor groove binders (*e.g.*, enediynes and lexitropsins), duocarmycins, taxanes (*e.g.*, paclitaxel and docetaxel), puromycins, vinca alkaloids, CC-1065, SN-38, topotecan, morpholino-doxorubicin, rhizoxin, cyanomorpholino-doxorubicin, echinomycin, combretastatin, netropsin, epothilone A and B, estramustine, cryptophysins, cemadotin, maytansinoids,
10 discodermolide, eleutherobin, and mitoxantrone.

 In a specific embodiment, the cytotoxic or cytostatic agent is auristatin E (dolastatin-10) or a derivative thereof (*e.g.* an ester formed between auristatin E and a keto acid). Other typical auristatin derivatives include AFP, MMAR, and MMAE. The synthesis and
15 structure of auristatin E and its derivatives are described in U.S. Patent Application Publication No. 20030083263; PCT/US03/24209; PCT/US02/13435; and U.S. Patent Nos. 6,323,315; 6,239,104; 6,034,065; 5,780,588; 5,665,860; 5,663,149; 5,635,483; 5,599,902; 5,554,725; 5,530,097; 5,521,284; 5,504,191; 5,410,024; 5,138,036; 5,076,973; 4,986,988; 4,978,744; 4,879,278; 4,816,444; and 4,486,414.

20 In a specific embodiment anti-GPNMB antibody 1.15.1 was coupled to monomethylauristatin E via intracellular protease-sensitive valine-citrulline peptide linker (vcMMAE). Methods for making the immunoconjugate can be found in Doronina S.O. et al, 2003 Nature Biotechnology 21(7):778-794.

 Techniques for conjugating therapeutic agents to proteins, and in particular,
25 antibodies are known in the art (see, *e.g.* Arnon *et al.*, 1985 in Monoclonal Antibodies and Cancer Therapy, Reisfeld *et al.* eds., Alan R. Liss, Inc., 1985; Hellstrom *et al.*, 1987 in Controlled Drug Delivery, Robinson *et al.* eds., Marcel Dekker, Inc., 2nd ed. 1987; Thorpe 1985, in Monoclonal Antibodies '84: Biological and Clinical Applications, Pinchera *et al.* eds., EDITOR, 1985; Monoclonal Antibodies for Cancer Detection and Therapy, Baldwin *et al.* eds., Academic Press 1985; and Thorpe *et al.*, 1982, Immunol. Rev. 62:119-58).
30

 In certain embodiments of the invention, anti-GPNMB antibodies binding to GPNMB expressing cells, are internalized and accumulate in the cell. Thereby anti-GPNMB antibody immunoconjugates accumulate in GPNMB expressing cells. Typically when the

anti-GPNMB antibody immunoconjugate is internalized, the agent is preferentially active. Alternatively, anti GPNMB immunoconjugates are not internalized and the drug is effective to deplete or inhibit GPNMB expressing cells by binding to the cell membrane. The therapeutic agent can be conjugated in a manner that reduces its activity unless it is cleaved
5 off the antibody (e.g. by hydrolysis or by a cleaving agent). In this case, the agent can be attached to the antibody or derivative thereof with a cleavable linker that is sensitive to cleavage in the intracellular environment of the target but is not substantially sensitive to the extracellular environment, such that the conjugate is cleaved from the antibody or derivative thereof when it is internalized by the GPNMB expressing cell (e.g. in the endosomal or, for
10 example by virtue of pH sensitivity or protease sensitivity, in the lysosomal environment or in a caveolea).

A therapeutic agent of the immunoconjugate can be charged relative to the plasma membrane (e.g. polarized or net charge relative to the plasma membrane), thereby further minimizing the ability of the agent to cross the plasma membrane once internalized by a
15 cell.

The anti-GPNMB antibody immunoconjugate can comprise a linker region between the therapeutic agent and the antibody. The linker can be cleavable under intracellular conditions, such that cleavage of the linker releases the therapeutic agent from the antibody in the intracellular environment. The linker can be, e.g. a peptidyl linker that is cleaved by
20 an intracellular peptidase or protease enzyme, including but not limited to a lysosomal or endosomal protease. Often the peptidyl linker is at least two amino acids long or at least three amino acids long. Cleaving agents can include cathepsins and D and plasmin, all of which are known to hydrolyze dipeptide drug derivatives resulting in the release of active drug inside target cells (see Dubowchik and Walker, 1999 Pharm. Therapeutics 83:67-123).
25 Other linkers are described e.g. in U.S. Patent No. 6,214,345.

Linkers can be pH-sensitive can often be hydrolyzable under acidic conditions such as is found in the lysosome (see e.g. U.S. Patent Nos. 5,122,368; 5,824,805; 5,622,929; Dubowchik and Walker, 1999 Pharm. Therapeutics 83:67-123; Neville *et al.*, 1989 Biol. Chem. 264:14653-14661). Such linkers are relatively stable under neutral pH conditions,
30 such as those in the blood, but are unstable at below pH 5.5 or 5.0, the pH of the lysosome. Linkers can be cleavable under reducing conditions (e.g. a disulfide linker) (see e.g., Thorpe *et al.*, 1987 Cancer Res. 47:5924-5931; Wawrzynczak *et al.*, In Immunoconjugates: Antibody Conjugates in Radioimmunity and Therapy of Cancer, C.W. Vogel ed, Oxford U.

Press, 1987; U.S. Patent No. 4,880,935). The linker can be a malonate linker (Johnson *et al.*, 1995, Anticancer Res. 15:1387-93), a maleimidobenzoyl linker (Lau *et al.*, 1995, Bioorg-Med-Chem. 3(10):1299-1304) or a 3'-N-amide analog (Lau *et al.*, 1995, Bioorg-Med-Chem.3(10):1305-1312).

5 Prophylactic and Therapeutic Uses of the Present Invention

 The antibodies of the invention can act as either agonists or antagonists of GPNMB, depending on the methods of their use. The antibodies can be used to prevent, diagnose, or treat medical disorders in a subject, especially in humans. Antibodies of the invention can also be used for isolating GPNMB or GPNMB-expressing cells. Furthermore, the antibodies
10 can be used to treat a subject at risk of or susceptible to a disorder or having a disorder associated with aberrant GPNMB expression or function. Antibodies of the invention can be used to detect GPNMB in such subjects.

 The present invention provides methods for treating and/or preventing a disease or disorder associated with overexpression of GPNMB and/or cell hyperproliferative disorders,
15 particularly cancer, in a subject comprising administering an effective amount of a composition that can target cells expressing GPNMB, and inhibiting the GPNMB expression or function, and/or having therapeutic or prophylactic effects on the hyperproliferative cell disease. In one embodiment, the method of the invention comprises administering to a subject a composition comprising an immunoconjugate that comprises an
20 antibody of the invention and a cytotoxic agent against the hyperproliferative cell disease. In another embodiment, the method of the invention comprises administering to a subject in need thereof a composition comprising a naked IgG1 antibody of the invention and one or more immunomodulators. In yet another embodiment, the method of the invention comprises administering to a subject in need thereof a composition comprising a single
25 chain Fv antibody (anti-GPNMB) conjugated to a cytotoxic agent, or a composition comprising a bispecific antibody that have a single chain anti-GPNMB antibody component and a anti-CD3 antibody component. In a preferred embodiment, the hyperproliferative cell disease is cancer. More preferably, the cancer is melanoma, or a cancer of the CNS system, such as astrocytoma, glioblastoma, medulloblastoma, or neoplastic meningitis.

30 The present invention provides therapies comprising administering one of more antibodies of the invention and compositions comprising said antibodies to a subject, preferably a human subject, for preventing and/or treating a disorder characterized by or associated with aberrant expression and/or activity of GPNMB or a symptom thereof. In

one embodiment, the invention provides a method of preventing or treating a disorder characterized by or associated with aberrant expression and/or activity of GPNMB or a symptom thereof, said method comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention. In certain embodiments, an effective amount of one or more immunoconjugates comprising one or more antibodies of the invention is administered to a subject in need thereof to prevent or treat a disorder characterized by or associated with aberrant expression and/or activity of GPNMB or a symptom thereof.

The invention also provides methods of preventing or treating a disorder characterized by or associated with aberrant expression and/or activity of GPNMB or a symptom thereof, said methods comprising administering to a subject in need thereof one or more of the antibodies of the invention and one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents) other than antibodies of the invention. The prophylactic or therapeutic agents of the combination therapies of the invention can be administered sequentially or concurrently. In a specific embodiment, the combination therapies of the invention comprise an effective amount of one or more antibodies of the invention and an effective amount of at least one other therapy (*e.g.*, prophylactic or therapeutic agent) which has a different mechanism of action than said antibodies. In certain embodiments, the combination therapies of the present invention improve the prophylactic or therapeutic effect of one or more antibodies of the invention by functioning together with the antibodies to have an additive or synergistic effect. In certain embodiments, the combination therapies of the present invention reduce the side effects associated with the therapies (*e.g.*, prophylactic or therapeutic agents).

The prophylactic or therapeutic agents of the combination therapies can be administered to a subject, preferably a human subject, in the same pharmaceutical composition. Alternatively, the prophylactic or therapeutic agents of the combination therapies can be administered concurrently to a subject in separate pharmaceutical compositions. The prophylactic or therapeutic agents may be administered to a subject by the same or different routes of administration.

In a specific embodiment, a pharmaceutical composition comprising one or more antibodies of the invention described herein is administered to a subject, preferably a human, to prevent and/or treat a disorder characterized by or associated with aberrant expression and/or activity of GPNMB or a symptom thereof. In accordance with the

invention, pharmaceutical compositions of the invention may also comprise one or more therapies (*e.g.*, prophylactic or therapeutic agents), other than antibodies of the invention.

The antibodies of the invention may also be used to detect the presence of GPNMB in biological samples (in diagnostic methods or use as an efficacy marker). The amount of GPNMB detected may be correlated with the expression level of GPNMB, which, in turn, is correlated with the disease, tumor type, tumor burden or stage using methods known in the art (see for example recommendations of the AAPS Ligand Binding Assay Bioanalytical Focus Group (LBABFG) *Pharm Res.* 2003 Nov;20(11):1885-900). Detection methods that employ antibodies are well known in the art and include, for example, ELISA, radioimmunoassay, immunoblot, Western blot, IHC, immunofluorescence, immunoprecipitation. The antibodies may be provided in a diagnostic kit that incorporates one or more of these techniques to detect GPNMB. Such a kit may contain other components, packaging, instructions, or other material to aid the detection of the protein. In a specific embodiment, the antibodies of the invention are conjugated to a radioactive isotope, and are injected to a subject to detect cells that overexpressing GPNMB.

Where the antibodies are intended for diagnostic purposes, it may be desirable to modify them, for example, with a ligand group (such as biotin) or a detectable marker group (such as a fluorescent group, a radioisotope or an enzyme). If desired, the antibodies of the invention may be labeled using conventional techniques. Suitable detectable labels include, for example, fluorophores, chromophores, radioactive atoms, electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase can be detected by its ability to convert tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. For detection, suitable binding partners include, but are not limited to, biotin and avidin or streptavidin, IgG and protein A, and the numerous receptor-ligand couples known in the art. Other permutations and possibilities will be readily apparent to those of ordinary skill in the art, and are considered as equivalents within the scope of the instant invention.

Antibodies of the invention can be used in screening methods to identify inhibitors of GPNMB effective as therapeutics. In such a screening assay, a first binding mixture is formed by combining GPNMB and an antibody of the invention; and the amount of binding in the first binding mixture (M_0) is measured. A second binding mixture is also formed by combining GPNMB, the antibody, and the compound or agent to be screened, and the amount of binding in the second binding mixture (M_1) is measured. A compound to be

tested may be another anti-GPNMB antibody. The amounts of binding in the first and second binding mixtures are then compared, for example, by calculating the M_1/M_0 ratio. The compound or agent is considered to be capable of modulating a GPNMB-associated responses if a decrease in binding in the second binding mixture as compared to the first binding mixture is observed. The formulation and optimization of binding mixtures is within the level of skill in the art, such binding mixtures may also contain buffers and salts necessary to enhance or to optimize binding, and additional control assays may be included in the screening assay of the invention. Compounds found to reduce the GPNMB-antibody binding by at least about 10% (*i.e.*, $M_1/M_0 < 0.9$), preferably greater than about 30% may thus be identified and then, if desired, secondarily screened for the capacity to ameliorate a disorder in other assays or animal models as described below. The strength of the binding between GPNMB and an antibody can be measured using, for example, an enzyme-linked immunoadsorption assay (ELISA), radio-immunoassay (RIA), surface plasmon resonance-based technology (*e.g.*, Biacore), all of which are techniques well known in the art.

The compound may then be tested in vitro as described in the Examples, *infra*.

Dosage and Frequency of Administration

The amount of a prophylactic or therapeutic agent or a composition of the invention which will be effective in the prevention and/or treatment of a disorder associated with or characterized by aberrant expression and/or activity of GPNMB can be determined by standard clinical methods. For example, the dosage of the composition which will be effective in the treatment and/or prevention of cancer can be determined by administering the composition to an animal model. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. Preliminary doses as, for example, determined according to animal tests, and the scaling of dosages for human administration is performed according to art-accepted practices. Toxicity and therapeutic efficacy can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The data obtained from the cell culture assays or animal studies can be used in formulating a range of dosage for use in humans. Therapeutically effective dosages achieved in one animal model can be converted for use in another animal, including humans, using conversion factors known in the art (*see, e.g., Freireich et al. (1966) Cancer Chemother. Reports, 50(4): 219-244*).

Selection of the preferred effective dose can be determined (*e.g.*, via clinical trials) by a skilled artisan based upon the consideration of several factors which will be known to

one of ordinary skill in the art. Such factors include the disease to be treated or prevented, the symptoms involved, the patient's body mass, gender, immune status and other factors known by the skilled artisan to reflect the accuracy of administered pharmaceutical compositions. Suitable regimens can be selected by one skilled in the art by considering
5 such factors and by following, for example, dosages reported in literature and recommended in the Physician's Desk Reference (59th ed., 2005).

The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the cancer, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be
10 extrapolated from dose-response curves derived from in vitro or animal model test systems.

For other cancer therapeutic agents administered to a patient, the typical doses of various cancer therapeutics are known in the art. Given the invention, certain preferred embodiments will encompass the administration of lower dosages in combination treatment regimens than dosages recommended for the administration of single agents.

15 In a specific embodiment, the dosage of an antibody or an immunoconjugate comprising an antibody of the invention administered to prevent and/or treat a disorder associated with or characterized by aberrant expression and/or activity of GPNMB (*e.g.*, cancer) in a patient is 30 mg/kg or less, 25 mg/kg or less, 20 mg/kg or less, 15 mg/kg or less, preferably 12 mg/kg or less, 11 mg/kg or less, 10 mg/kg or less, 9 mg/kg or less, 8
20 mg/kg or less, 7 mg/kg or less, 6 mg/kg or less, 5 mg/kg or less, 4 mg/kg or less, 3 mg/kg or less, 2 mg/kg or less, or 1 mg/kg or less of a patient's body weight. In another embodiment, the dosage of an antibody or an immunoconjugate of the invention administered to prevent and/or treat a disorder associated with or characterized by aberrant expression and/or activity of GPNMB (*e.g.*, cancer) in a patient is a unit dose of about 0.01 mg/kg to about 20
25 mg/kg, about 0.1 mg/kg to about 10 mg/kg, about 0.1 mg/kg to about 8 mg/kg, about 0.1 mg/kg to about 7 mg/kg, about 0.1 mg/kg to about 6 mg/kg, about 0.1 mg/kg to about 5 mg/kg, about 0.1 mg/kg to about 4 mg/kg, preferably, about 0.1 mg/kg to about 3 mg/kg, about 0.2 mg/kg to 3 mg/kg, about 0.3 mg/kg to about 3 mg/kg, about 0.4 mg/kg to about 3 mg/kg, about 0.6 mg/kg to about 3 mg/kg, about 0.8 mg/kg to about 3 mg/kg, about 0.1
30 mg/kg to 2 mg/kg, about 0.1 mg/kg to 1 mg/kg. In certain embodiments, the dosage of an antibody or an immunoconjugate comprising an antibody of the invention administered to prevent and/or treat a disorder associated with or characterized by aberrant expression and/or activity of GPNMB (*e.g.*, cancer) in a patient is a unit dose of about 0.1 mg/kg, about

0.2 mg/kg, about 0.4 mg/kg, about 0.6 mg/kg, about 0.8 mg/kg, about 1.1 mg/kg, or about 1 mg/kg.

In certain embodiments, a subject is administered one or more doses of an effective amount of one or more antibodies or immunoconjugates of the invention to prevent and/or
5 treat a disorder associated with or characterized by aberrant expression and/or activity of GPNMB, wherein the dose of an effective amount of said antibodies, immunoconjugates, compositions, or combination therapies reduces and/or inhibits proliferation of cancerous cells by at least 20% to 25%, preferably at least 25% to 30%, at least 30% to 35%, at least
35% to 40%, at least 40% to 45%, at least 45% to 50%, at least 50% to 55%, at least 55% to
10 60%, at least 60% to 65%, at least 65% to 70%, at least 70% to 75%, at least 75% to 80%, at least 80 to 85%, at least 85% to 90%, at least 90% to 95%, or at least 95% to 98% relative to a control such as PBS in an in vitro and/or in vivo assay well-known in the art.

In other embodiments, a subject is administered one or more doses of an effective amount of one or more antibodies or immunoconjugates of the invention to prevent and/or
15 treat a disorder associated with or characterized by aberrant expression and/or activity of GPNMB, wherein the dose of an effective amount achieves a serum titer of at least 0.1 $\mu\text{g/mL}$, at least 0.5 $\mu\text{g/mL}$, at least 1 $\mu\text{g/mL}$, at least 2 $\mu\text{g/mL}$, at least 5 $\mu\text{g/mL}$, at least 6 $\mu\text{g/mL}$, at least 10 $\mu\text{g/mL}$, at least 15 $\mu\text{g/mL}$, at least 20 $\mu\text{g/mL}$, at least 25 $\mu\text{g/mL}$, at least 50 $\mu\text{g/mL}$, at least 100 $\mu\text{g/mL}$, at least 125 $\mu\text{g/mL}$, at least 150 $\mu\text{g/mL}$, at least 175 $\mu\text{g/mL}$,
20 at least 200 $\mu\text{g/mL}$, at least 225 $\mu\text{g/mL}$, at least 250 $\mu\text{g/mL}$, at least 275 $\mu\text{g/mL}$, at least 300 $\mu\text{g/mL}$, at least 325 $\mu\text{g/mL}$, at least 350 $\mu\text{g/mL}$, at least 375 $\mu\text{g/mL}$, or at least 400 $\mu\text{g/mL}$ of the antibodies of the invention. In yet other embodiments, a subject is administered a dose of an effective amount of one or more antibodies or immunoconjugates of the invention to achieve a serum titer of at least 0.1 $\mu\text{g/mL}$, at least 0.5 $\mu\text{g/mL}$, at least 1 $\mu\text{g/mL}$, at least, 2
25 $\mu\text{g/mL}$, at least 5 $\mu\text{g/mL}$, at least 6 $\mu\text{g/mL}$, at least 10 $\mu\text{g/mL}$, at least 15 $\mu\text{g/mL}$, at least 20 $\mu\text{g/mL}$, at least 25 $\mu\text{g/mL}$, at least 50 $\mu\text{g/mL}$, at least 100 $\mu\text{g/mL}$, at least 125 $\mu\text{g/mL}$, at least 150 $\mu\text{g/mL}$, at least 175 $\mu\text{g/mL}$, at least 200 $\mu\text{g/mL}$, at least 225 $\mu\text{g/mL}$, at least 250 $\mu\text{g/mL}$, at least 275 $\mu\text{g/mL}$, at least 300 $\mu\text{g/mL}$, at least 325 $\mu\text{g/mL}$, at least 350 $\mu\text{g/mL}$, at least 375 $\mu\text{g/mL}$, or at least 400 $\mu\text{g/mL}$ of the antibodies and a subsequent dose of an
30 effective amount of one or more antibodies or immunoconjugates of the invention is administered to maintain a serum titer of at least 0.1 $\mu\text{g/mL}$, at least 0.5 $\mu\text{g/mL}$, at least 1 $\mu\text{g/mL}$, at least, 2 $\mu\text{g/mL}$, at least 5 $\mu\text{g/mL}$, at least 6 $\mu\text{g/mL}$, at least 10 $\mu\text{g/mL}$, at least 15 $\mu\text{g/mL}$, at least 20 $\mu\text{g/mL}$, at least 25 $\mu\text{g/mL}$, at least 50 $\mu\text{g/mL}$, at least 100 $\mu\text{g/mL}$, at least

125 µg/mL, at least 150 µg/mL, at least 175 µg/mL, at least 200 µg/mL, at least 225 µg/mL, at least 250 µg/mL, at least 275 µg/mL, at least 300 µg/mL, at least 325 µg/mL, at least 350 µg/mL, at least 375 µg/mL, or at least 400 µg/mL. In accordance with these embodiments, a subject may be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more subsequent doses.

5 In a specific embodiment, the invention provides methods of preventing and/or treating a disorder associated with or characterized by aberrant expression and/or activity of GPNMB, said method comprising administering to a subject in need thereof a unit dose of at least 0.01mg/kg, at least 0.1mg/kg, at least 0.2mg/kg, at least 0.4mg/kg, at least 0.6mg/kg, at least 0.8mg/kg, at least 1mg/kg, or at least 1.1mg/kg of one or more antibodies
10 or immunoconjugates of the invention. In another embodiment, the invention provides methods of preventing and/or treating a disorder associated with or characterized by aberrant expression and/or activity of GPNMB, said method comprising administering to a subject in need thereof a unit dose of at least 0.01 mg/kg, at least 0.1 mg/kg, at least 0.2 mg/kg, at least 0.4 mg/kg, at least 0.6 mg/kg, at least 0.8 mg/kg, at least 1 mg/kg, or at least
15 1.1 mg/kg of one or more antibodies or immunoconjugates of the invention once every 7 days, preferably, once every 10 days, once every 12 days, once every 14 days, once every 16 days, once every 18 days, once every three weeks, or once a month. In a preferred embodiment, an immunoconjugate of the instant invention is administered at a unit dose of about 0.1 mg/kg, about 0.2 mg/kg, about 0.4 mg/kg, about 0.6 mg/kg, about 0.8 mg/kg,
20 about 1.1 mg/kg, or about 1 mg/kg once every 10 to 20 days with 2 to 4 cycles.

The present invention provides methods of preventing and/or treating a disorder associated with or characterized by aberrant expression and/or activity of GPNMB, said method comprising: (a) administering to a subject in need thereof one or more doses of a prophylactically or therapeutically effective amount of one or more antibodies or
25 immunoconjugates of the invention; and (b) monitoring the plasma level/concentration of the said administered antibody or antibodies in said subject after administration of a certain number of doses of the said antibody or antibodies. Moreover, preferably, said certain number of doses is 1, 2, 3, 4, 5, 6, 7, or 8 doses of a prophylactically or therapeutically effective amount one or more antibodies or immunoconjugates of the invention.

30 In a specific embodiment, the invention provides a method of preventing and/or treating a disorder associated with or characterized by aberrant expression and/or activity of GPNMB, said method comprising: (a) administering to a subject in need thereof a dose of at least 0.1 mg/kg (preferably at least at least 0.2 mg/kg, at least 0.4 mg/kg, at least 0.6 mg/kg,

at least 0.8 mg/kg, at least 1 mg/kg, or at least 1.1 mg/kg) of one or more antibodies or immunoconjugates of the invention; and (b) administering one or more subsequent doses to said subject when the plasma level of the antibody or antibodies administered in said subject is less than 0.1 µg/mL, preferably less than 0.25 µg/mL, less than 0.5 µg/mL, less than 0.75 µg/mL, or less than 1 µg/mL. In another embodiment, the invention provides a method of preventing and/or treating a disorder associated with or characterized by aberrant expression and/or activity of GPNMB, said method comprising: (a) administering to a subject in need thereof one or more doses of at least at least 0.1 mg/kg (preferably at least at least 0.2 mg/kg, at least 0.4 mg/kg, at least 0.6 mg/kg, at least 0.8 mg/kg, at least 1 mg/kg, or at least 1.1 mg/kg) of one or more antibodies of the invention; (b) monitoring the plasma level of the administered antibody or antibodies of the invention in said subject after the administration of a certain number of doses; and (c) administering a subsequent dose of the antibody or antibodies of the invention when the plasma level of the administered antibody or antibodies in said subject is less than 0.1 µg/mL, preferably less than 0.25 µg/mL, less than 0.5 µg/mL, less than 0.75 µg/mL, or less than 1 µg/mL. Preferably, said certain number of doses is 1, 2, 3, 4, 5, 6, 7, or 8 doses of an effective amount of one or more antibodies or immunoconjugates of the invention.

Therapies (*e.g.*, prophylactic or therapeutic agents), other than antibodies or immunoconjugates of the invention, which have been or are currently being used to prevent and/or treat a disorder associated with or characterized by aberrant expression and/or activity of GPNMB can be administered in combination with one or more antibodies or immunoconjugates of the invention according to the methods of the invention to treat and/or prevent a disorder associated with or characterized by aberrant expression and/or activity of GPNMB. Preferably, the dosages of prophylactic or therapeutic agents used in combination therapies of the invention are lower than those which have been or are currently being used to prevent and/or treat a disorder associated with or characterized by aberrant expression and/or activity of GPNMB.

In various embodiments, the therapies (*e.g.*, prophylactic or therapeutic agents) are administered less than 5 minutes apart, less than 30 minutes apart, 1 hour apart, at about 1 hour apart, at about 1 to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10

hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, at about 12 hours to 18 hours apart, 18 hours to 24 hours apart, 24 hours to 36 hours apart, 36 hours to 48 hours apart, 48 hours to 52 hours apart, 52 hours to 60 hours apart, 60 hours to 72 hours apart, 72 hours to 84 hours apart, 84 hours to 96 hours apart, or 96 hours to 120 hours part. In preferred embodiments, two or more therapies are administered within the same patient visit.

In certain embodiments, one or more antibodies of the invention and one or more other therapies (e.g., prophylactic or therapeutic agents) are cyclically administered. Cycling therapy involves the administration of a first therapy (e.g., a first prophylactic or therapeutic agent) for a period of time, followed by the administration of a second therapy (e.g., a second prophylactic or therapeutic agent) for a period of time, optionally, followed by the administration of a third therapy (e.g., prophylactic or therapeutic agent) for a period of time and so forth, and repeating this sequential administration, i.e., the cycle in order to reduce the development of resistance to one of the therapies, to avoid or reduce the side effects of one of the therapies, and/or to improve the efficacy of the therapies.

Pharmaceutical Compositions and Methods of Administration

The disclosure provides compositions comprising anti-GPNMB antibodies. Such compositions may be suitable for pharmaceutical use and administration to patients. The compositions typically comprise one or more antibodies of the present invention and a pharmaceutically acceptable excipient. The phrase "pharmaceutically acceptable excipient" includes any and all solvents, dispersion media, coatings, antibacterial agents and antifungal agents, isotonic agents, and absorption delaying agents, and the like, that are compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. The compositions may also contain other active compounds providing supplemental, additional, or enhanced therapeutic functions. The pharmaceutical compositions may also be included in a container, pack, or dispenser together with instructions for administration.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Methods to accomplish the administration are known to those of ordinary skill in the art. The administration may, for example, be intravenous, intraperitoneal, intramuscular, intracavity, subcutaneous or transdermal. It may also be possible to obtain compositions which may be topically or orally administered, or which may be capable of transmission across mucous membranes.

Solutions or suspensions used for intradermal or subcutaneous application typically include one or more of the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol, or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. Such preparations may be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injection include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars; polyalcohols such as mannitol, sorbitol, and sodium chloride in the composition. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and/or by the use of surfactants. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate, and gelatin.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For oral administration, the antibodies can be combined with excipients and used in the form of tablets, troches, or capsules. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches, and the like can

contain any of the following ingredients, or compounds of a similar nature; a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration may be accomplished, for example, through the use of lozenges, nasal sprays, inhalers, or suppositories. For example, in case of antibodies that comprise the Fc portion, compositions may be capable of transmission across mucous membranes in intestine, mouth, or lungs (e.g., via the FcRn receptor-mediated pathway as described in U.S. Pat. No. 6,030,613). For transdermal administration, the active compounds may be formulated into ointments, salves, gels, or creams as generally known in the art. For administration by inhalation, the antibodies may be delivered in the form of an aerosol spray from pressured container or dispenser, which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

In certain embodiments, the presently disclosed antibodies are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Liposomal suspensions containing the presently disclosed antibodies can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

It may be advantageous to formulate oral or parenteral compositions in a dosage unit form for ease of administration and uniformity of dosage. The term "dosage unit form" as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to

produce the desired therapeutic effect in association with the required pharmaceutical carrier.

Toxicity and therapeutic efficacy of the composition of the invention can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compositions that exhibit large therapeutic indices are preferred.

For any composition used in the present invention, the therapeutically effective dose can be estimated initially from cell culture assays. Examples of suitable bioassays include DNA replication assays, clonogenic assays and other assays as, for example, described in the Examples. The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*i.e.*, the concentration of the antibody which achieves a half-maximal inhibition of symptoms). Circulating levels in plasma may be measured, for example, by high performance liquid chromatography. The effects of any particular dosage can be monitored by a suitable bioassay. The dosage lies preferably within a range of circulating concentrations with little or no toxicity. The dosage may vary depending upon the dosage form employed and the route of administration utilized.

Antibodies can be modified to become immunotoxins utilizing techniques that are well known in the art (*Vitetta 1993, Immunol Today 14:252; U.S. Patent No. 5,194,594*). Cytotoxic immunoconjugates are known in the art and have been used as therapeutic agents. Such immunoconjugates may for example, use maytansinoids (*US 6,441,163*), tubulin polymerization inhibitor, auristatin (*Mohammad et al, 1999 Int. J. Oncol 15(2):367-72; Doronina et al, 2003 Nature Biotechnology 21(7):778-784*), dolastatin derivatives (*Ogawa et al, 2001 Toxicol Lett. 121(2):97-106 21(3)778-784*), Mylotarg® (Wyeth Laboratories, Philadelphia, PA); maytansinoids (DM1), taxane or mertansine (ImmunoGen Inc.).

Immunoradiopharmaceuticals utilizing anti-GPNMB antibodies may be prepared utilizing techniques that are well known in the art (*Junghans et al. in Cancer Chemotherapy and Biotherapy 655-686 (2d edition, Chafner and Longo, eds., Lippincott Raven (1996); U.S. Patent Nos. 4,681,581, 4,735,210, 5,101,827, 5,102,990 (RE 35,500), 5,648,471, and 5,697,902*). Each of the immunotoxins and radiolabeled antibody molecules

selectively kill cells expressing GPNMB. Radiolabels are known in the art and have been used for diagnostic or therapeutic radioimmuno conjugates. Examples of radiolabels include, but are not limited to, the following: radioisotopes or radionuclides (e.g., ^3H , ^{14}C , ^{15}N , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I , ^{177}Lu , ^{105}Rh , Rhenium-186, Rhenium-188, Samarium-153, Copper-64, Scandium-47). For example, radionuclides which have been used in radioimmunoconjugate guided clinical diagnosis include, but are not limited to: ^{131}I , ^{125}I , ^{123}I , ^{99}Tc , ^{67}Ga , as well as ^{111}In . Antibodies have also been labeled with a variety of radionuclides for potential use in targeted immunotherapy (see *Peirersz et al.*, 1987). These radionuclides include, for example, ^{188}Re and ^{186}Re as well as ^{90}Y , and to a lesser extent ^{199}Au and ^{67}Cu . I-(131) (see for example U.S. Pat. No. 5,460,785). Radiotherapeutic chelators and chelator conjugates are known in the art (U.S. 4,831,175, 5,099,069, 5,246,692, 5,286,850, and 5,124,471).

EXAMPLES

The following examples, including the experiments conducted and results achieved are provided for illustrative purposes only and are not to be construed as limiting upon the present invention.

Example 1: Immunogen

Recombinant human GPNMB (SEQ ID NO:289), specifically the extra-cellular domain (ECD) was prepared for use as the immunogen. Generally, cDNA encoding the ECD of GPNMB with a C-terminus V5-HIS tag was transfected into HEK 293 cells, expressed and purified using cation exchange chromatography with a POROS HS 50 (Applied Biosystems, Foster City, CA). Sample was eluted with 1M NaCl at a pH of 5.5, followed by metal affinity chromatography (Pharmacia metal chelate 5 mL). The sample was eluted against a linear gradient from 10-500 mM imidazole over 10 CV (column volume). Dialysis occurred using 20mM Tris/ 50mM NaCl at pH 7.4 (2L x 2). The sample was then filtered through a 0.22 μm filter.

Example 2: Immunization

A preferred method for generating fully human antibodies uses XenoMouse® strains of mice which have been engineered to contain 245 kb and 190 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus (*Green et al.* 1994 *Nature Genetics* 7:13-21; *Mendez et al.* 1997 *Nature Genetics* 15:146-156;

Green and Jakobovits, 1998 J. Exp. Med. 188:483-495; U.S. Patent Nos. 6,162,963, 6,150,584, 6,114,598, 6,075,181, and 5,939,598.) In an alternative approach, the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of pieces (individual genes) from the Ig locus. Thus, one or more V_H genes, one or more D_H genes, one or more J_H genes, a mu constant region, and a second constant region (preferably a gamma constant region) are formed into a construct for insertion into an animal (*Taylor et al., 1992, Chen et al., 1993, Tuailon et al., 1993, Choi et al., 1993, Lonberg et al., (1994), Taylor et al., (1994), and Tuailon et al., (1995), Fishwild et al., (1996); U.S. Patent Nos. 5,545,807, 5,545,806, 5,625,825, 5,625,126, 5,633,425, 5,661,016, 5,770,429, 5,789,650, 5,814,318, 5,877,397, 5,874,299, 6,255,458, 5,591,669, 6,023,010, 5,612,205, 5,721,367, 5,789,215, 5,643,763, 5,981,175*). It is understood that the λx XenoMouse® may be used to generate anti-GPNMB antibodies utilizing lambda V regions. Such antibodies are within the scope of the invention.

Immunization

GPNMB-V5His immunogen (as prepared in Example 1) was used as an antigen. Monoclonal antibodies against GPNMB were developed by sequentially immunizing XenoMouse® mice (XenoMouse® XMG2 strain), Abgenix, Inc. Fremont, CA. XenoMouse® animals were immunized via footpad route for all injections. The total volume of each injection was 50 µl per mouse, 25 µl per footpad.

For cohort 1 (10 XMG2 mice), the initial immunization was with 10 µg of GPNMB-V5His admixed 1:1 (v/v) with 100 µg alum gel ("Adju-Phos": aluminum phosphate gel adjuvant, Superfos BIOSECTOR™ a/s, distributed by E.M. Sargent Pulp and Chemical Co., Clifton, NJ, cat. # 1452-250) per mouse. The subsequent five boosts were made with 5 µg of GPNMB-V5His admixed 1:1 (v/v) with 100 µg alum gel in pyrogen-free D-PBS. The seventh boost consisted of 5 µg of GPNMB-V5His admixed 1:1 (v/v) with TITERMAX GOLD® (Sigma; cat. # T2684). The eighth injection consisted of 5 µg of GPNMB-V5His admixed 1:1 v/v with 100 µg alum gel. A final boost was made with 5 µg GPNMB-V5His in pyrogen-free DPBS, without adjuvant. The XenoMouse® mice were immunized on days 0, 3, 6, 10, 14, 17, 23, and 27 for this protocol and fusions were performed on day 31. The bleed was made through Retro-Orbital Bleed procedure on day 21 after the sixth boost.

For cohort 2 (10 XMG2 mice), the initial immunization was with 10 µg of GPNMB-V5His admixed 1:1 (v/v) with 100 µg alum gel per mouse. The subsequent two boosts were made with 5 µg of GPNMB-V5His admixed 1:1 (v/v) with 100 µg alum gel in pyrogen-free D-PBS. The fourth boost consisted of 5 µg of GPNMB-V5His admixed 1:1 (v/v) with TITERMAX GOLD® (Sigma; cat. # T2684). The following fifth to seventh injection consisted of 5 µg of GPNMB-V5His admixed 1:1 v/v with 100 µg alum gel. The eighth injection and final boost was made with 5 µg GPNMB-V5His in pyrogen-free DPBS, without adjuvant. The XenoMouse® mice were immunized on days 0, 3, 7, 11, 14, 17, 22, 25 and 74. for this protocol and fusions were performed on day 78. The bleeds was made through Retro-Orbital Bleed procedure on day 21 after the sixth boost.

The footpad injection was performed by the following protocol using only the ventral surface of both hind limb paws. A solution was injected beneath the skin without piercing the muscle tissue by using an insulin 1/2 mL syringe with attached 28 or 30 gauge x 1/2" needle. The mouse to be injected was grasped by the loose fur along its neck and back so that it was immobilized and was turned over so the ventral side was accessible. The hind limb of the mouse was grasped and the needle was inserted (bevel side up) at the ankle, threading just under the skin until the needle tip reached the paw. The needle was inserted along the outside length of the hind foot carefully, to avoid the vein located towards the inner side of the foot. Once the tip of the needle reached the paw, the solution was injected slowly until resistance was felt or the designated volume had been dispensed. The needle was then withdrawn and the second hind foot injected in the same manner.

The following Table 4 provides the immunization schedule for the 2 groups of mice.

Table 4: Immunization Schedule of GPNMB Antigen: GPNMB-soluble at 043mg/mL

Target	Group No.	Mode of Immunization		No. of mice	Antigen	1st injection	2nd boost
GPNMB	1	Footpad		10	GPNMB-soluble	10ug/mouse Alum Gel Day 0	5ug/mouse Alum Gel Day 3

3rd boost	4th boost	5th boost	6th boost	Bleed	7th boost	8th boost	Fusion
5ug/mouse Alum Gel Day 6	5ug/mouse Alum Gel Day 10	5ug/mouse Alum Gel Day 14	5ug/mouse Alum Gel Day 17		5ug/mouse Titermax Gold Day 23	10ug/mouse D-PBS Day 27	

Target	group#	Mode of Immunization		# mice	Antigen	1st injection	2nd boost
GPNMB	2	Footpad		10	GPNMB-soluble	10ug/mouse Alum Gel Day 0	5ug/mouse Alum Gel Day 3

3rd boost	4th boost	5th boost	6th boost	Bleed	7th boost	8th boost	9th boost	Fusion
5ug/mouse Alum Gel Day 7	5ug/mouse Titermax Gold Day 11	5ug/mouse Alum Gel Day 14	5ug/mouse Alum Gel Day 17		5ug/mouse Alum Gel Day 22	10ug/mouse D-PBS Day 25	10ug/mouse D-PBS Day 100	

Selection of animals for harvest by titer

Anti- GPNMB antibody titers in the serum from immunized XenoMouse[®] mice were determined by ELISA. Briefly, three sets of ELISAs were set up. GPNMB (+NMB) at 1 µg/mL, GPNMB(-NMB) at 1 µg/mL, and NMB at 1 µg/mL were coated onto Costar Labcoat Universal Binding Polystyrene 96-well plates (Corning, Acton, MA) overnight at 4°C in Antigen Coating Buffer (0.1 M Carbonate Buffer, pH 9.6 NaHCO₃ (MW 84) 8.4 g/L). The next day, the plates were washed three times with washing buffer (0.05% Tween 20 in 1x PBS) using a Biotek plate washer. The plates were then blocked with 200 ul/well blocking buffer (0.5% BSA, 0.1% Tween 20, 0.01% Thimerosal in 1x PBS) and incubated at room temperature for 1 h. After the one-hour blocking, the plates were washed three times with washing buffer using a Biotek plate washer. Sera from either GPNMB immunized XenoMouse[®] mice or naïve XenoMouse[®] animals were titrated in 0.5% BSA/PBS buffer at 1:3 dilutions in duplicate from a 1:100 initial dilution. The last well was left blank. These plates were incubated at room temperature for 2 h, and the plates were then washed three times with washing buffer using a Biotek plate washer. A goat anti-human IgG Fc-specific horseradish peroxidase (HRP, Pierce, Rockford, IL) conjugated antibody was added at a final concentration of 1 µg/mL and incubated for 1 hour at room temperature. The plates were washed three times with washing buffer using a Biotek plate washer. After washing, the plates were developed with the addition of TMB chromogenic substrate (BioF_x BSTP-0100-01) for 10-20 min or until negative control wells start to show color. Then the ELISA was stopped by the addition of Stop Solution (650 nM Stop reagent for TMB (BioF_x BSTP-0100-01), reconstituted with 100 mL H₂O per bottle). The specific

titer of each XenoMouse[®] animal was determined from the optical density at 650 nm and is shown in Tables 2 and 3 below. The titer value is the reciprocal of the greatest dilution of sera with an OD reading two-fold that of background. Therefore, the higher the number, the greater was the humoral immune response to GPNMB. The results are provided in Table 5.

5 Table 5: XENOMOUSE[®] Anti-GPNMB Serum titers

Group 1 mice, fusion on Day 21 after 6 inj.			
Mouse ID	Reactivity to GPNMB	Reactivity to GPNMB (+GPNMB)	Reactivity to GPNMB
	Titers via hIgG	Titers via hIgG	Titers via hIgG
1-1	20,000	5,000	225
1-2	5,000	800	200
1-3	35,000	7,500	225
1-4	75,000	22,000	225
1-5	8,000	2,000	325
1-6	6,000	800	1800
1-7	22,000	7,500	225
1-8	6,000	2,000	200
1-9	7,000	2,000	75
1-10	22,000	7,500	200
1-NC1	<100	<100	<100
1-NC2	<100	<100	<100
Group 2 mice, bled on Day 21 after 6 inj.			
Mouse ID	Reactivity to GPNMB (-GPNMB)	Reactivity to GPNMB (+GPNMB)	Reactivity to GPNMB
	Titers via hIgG	Titers via hIgG	Titers via hIgG
2-1	100,000	2,600	50
2-2	8,000	2,600	50
2-3	15,000	4,000	50
2-4	7,000	2,200	75
2-5	22,000	6,500	250
2-6	60,000	22,000	60
2-7	19,000	7,000	50
2-8	5,000	1,200	50
2-9	16,000	3,500	110
2-10	12,000	5,000	110
2-NC1	<100	<100	<100
2-NC2	<100	<100	<100

Pooled anti-GPNMB sera from immunized animals was also evaluated by FACS for reactivity to UACC-62, SF539, SKMEL5, U87MG, and LOX1MVI cell lines. Pooled sera were tested at 1:10, 1:100 and 1:500 compared to Anti-IL13 serum (control) and prebleeds diluted at 1:10, 1:100 (control).

5 **Example 3: Antibodies**

Hybridoma cell lines were generated from immunized mice demonstrated to have anti-GPNMB titers using standard techniques (see Mendez et al, 1997, Nat Genet. 15:146-156).

10 Immunized mice were sacrificed by cervical dislocation, and the lymph nodes were harvested and pooled from each cohort. The lymphoid cells were dissociated by grinding in DMEM to release the cells from the tissues, and the cells were suspended in DMEM. The cells were counted, and 0.9 mL DMEM per 100 million lymphocytes was added to the cell pellet to resuspend the cells gently but completely. Using 100 μ L of CD90+ magnetic beads per 100 million cells, the cells were labeled by incubating the cells with the magnetic beads
15 at 4°C for 15 minutes. The magnetically-labeled cell suspension containing up to 10^8 positive cells (or up to 2×10^9 total cells) was loaded onto a LS+ column and the column washed with DMEM. The total effluent was collected as the CD90-negative fraction (most of these cells were expected to be B cells).

The fusion was performed by mixing washed enriched B cells from above and
20 nonsecretory myeloma P3X63Ag8.653 cells purchased from ATCC, cat.# CRL 1580 (Kearney et al, J. Immunol. 123, 1979, 1548-1550) at a ratio of 1:1. The cell mixture was gently pelleted by centrifugation at 800 g. After complete removal of the supernatant, the cells were treated with 2-4 mL of Pronase solution (CalBiochem, cat. # 53702; 0.5 mg/mL in PBS) for no more than 2 minutes. Then 3-5 mL of FBS was added to stop the enzyme
25 activity and the suspension was adjusted to 40 mL total volume using electro cell fusion solution, ECFS (0.3 M Sucrose, Sigma, Cat# S7903, 0.1 mM Magnesium Acetate, Sigma, Cat# M2545, 0.1 mM Calcium Acetate, Sigma, Cat# C4705). The supernatant was removed after centrifugation and the cells were resuspended in 40 mL ECFS. This wash step was repeated and the cells again were resuspended in ECFS to a concentration of 2×10^6
30 cells/mL.

Electro-cell fusion was performed using a fusion generator, model ECM2001, Genetronic, Inc., San Diego, CA. The fusion chamber size used was 2.0 mL, using the Abgenix, Inc. optimum instrument settings to do ECF.

After ECF, the cell suspensions were carefully removed from the fusion chamber under sterile conditions and transferred into a sterile tube containing the same volume of Hybridoma Culture Medium (DMEM (JRH Biosciences), 15% FBS (Hyclone), supplemented with L-glutamine, pen/strep, OPI (oxaloacetate, pyruvate, bovine insulin) (all from Sigma) and IL-6 (Boehringer Mannheim)). The cells were incubated for 15-30 minutes at 37°C, and then centrifuged at 400 g (1000 rpm [but in what rotor? Otherwise, leave out the rpm]) for five minutes. The cells were gently resuspended in a small volume of Hybridoma Selection Medium (Hybridoma Culture Medium supplemented with 0.5x HA (Sigma, cat. # A9666)), and the volume was adjusted appropriately with more Hybridoma Selection Medium, based on a final plating of 5×10^6 B cells total per 96-well plate and 200 μ L per well. The cells were mixed gently and pipetted into 96-well plates and allowed to grow. On day 7 or 10, one-half the medium was removed, and the cells were re-fed with Hybridoma Selection Medium.

After 14 days of culture, hybridoma supernatants were screened for GPNMB specific monoclonal antibodies. In the Primary screen, the ELISA plates (Fisher, Cat. No. 12-565-136) were coated with 50 μ L/well of GPNMB (1 μ g/mL) in Coating Buffer (0.1 M Carbonate Buffer, pH 9.6, NaHCO_3 8.4 g/L), then incubated at 4°C overnight. After incubation, the plates were washed with Washing Buffer (0.05% Tween 20 in PBS) three times. 200 μ L/well Blocking Buffer (0.5% BSA, 0.1% Tween 20, 0.01% Thimerosal in 1x PBS) were added and the plates were incubated at room temperature for 1 h. After incubation, the plates were washed with Washing Buffer three times. Aliquots (50 μ L/well) of hybridoma supernatants and positive and negative controls were added, and the plates were incubated at room temperature for 2 h. The positive control used throughout was serum from the relevant GPNMB immunized XenoMouse® mouse and the negative control was serum from the KLH-immunized relevant strain of XenoMouse® mouse. After incubation, the plates were washed three times with Washing Buffer. 100 μ L/well of detection antibody goat anti-huIgGfc-HRP (Caltag, Cat. No. H10507, using concentration was 1:2000 dilution) was added and the plates were incubated at room temperature for 1 hour. After incubation, the plates were washed three times with Washing Buffer. 100 μ L/well of TMB (BioFX Lab. Cat. No. TMSK-0100-01) was added, and the plates were allowed to develop for about 10 minutes (until negative control wells barely started to show color). 50 μ L/well stop solution (TMB Stop Solution (BioFX Lab. Cat. No. STPR-0100-01))

was then added and the plates were read on an ELISA plate reader at a wavelength of 450 nm.

The old culture supernatants from the positive hybridoma cells growth wells based on primary screen were removed completely and the IL-1b positive hybridoma cells were suspended with fresh hybridoma culture medium and were transferred to 24-well plates. After 2 days in culture, these supernatants were ready for a secondary confirmation screen. In the secondary confirmation screen, the positives in the first screening were screened in GPNMB binding ELISA described as above, and two sets of detective system for the secondary confirmation ELISA, one set for hIgG detection, one set for human Ig kappa light chain detection (goat anti-hIg kappa-HRP, Southern Biotechnology, Cat. No. 2060-05) in order to demonstrate fully human composition for both heavy and light chains. The two sets of ELISA procedures were identical to the descriptions above except the three different detection antibodies were used separately. All positive hits from the secondary confirmation ELISA assay were counter screened for binding to immunogen by ELISA in order to exclude those that cross-react with IL-1a. The ELISA plates (Fisher, Cat. No. 12-565-136) were coated with 50 μ L/well of irrelevant V5His-fusion protein, 1 μ g/mL in Coating Buffer (0.1 M Carbonate Buffer, pH 9.6, NaHCO₃ 8.4 g/L), then incubated at 4°C overnight. The remaining procedures were identical to the descriptions above. There are 33 fully human GPNMB specific monoclonal antibodies that were generated.

Hybridoma supernatants were screened for binding to GPNMB by ELISA as described above in Example 2. Results are shown in Table 6.

Table 6. Hybridoma anti-GPNMB activity.

	3 μ g/mL	1 μ g/mL	333 ng/mL	111 ng/mL	37 ng/mL	12.3 ng/mL
	Avg OD	Avg OD	Avg OD	Avg OD	Avg OD	Avg OD
1.2.2	0.763	0.499	0.356	0.199	0.094	0.049
1.7.3	1.003	0.871	0.760	0.451	0.239	0.094
1.15.1	1.159	1.051	0.902	0.701	0.381	0.168
1.16.2	0.036	0.015	0.010	0.008	0.008	0.007
2-3	1.282	1.204	0.963	0.713	0.359	0.179
2-6	1.254	1.295	1.092	0.875	0.443	0.183
2-7	0.827	0.719	0.680	0.494	0.308	0.156
2-8	0.921	0.635	0.229	0.109	0.056	0.028
2-10	1.095	1.066	0.849	0.583	0.272	0.132
2-15	0.601	0.568	0.578	0.395	0.246	0.127
2-16	0.359	0.173	0.068	0.032	0.017	0.011
2-17	0.053	0.019	0.010	0.009	0.008	0.011

2-22	0.714	0.707	0.538	0.355	0.171	0.068
2-24	0.060	0.042	0.028	0.023	0.016	0.017
Isotype control	0.009	0.008	0.009	0.009	0.009	0.011
Irrelevant Antibody	0.009	0.008	0.012	0.013		
Secondary Ab control	0.011					
Anti-V5 Ab control	3.066					

Certain Hybridoma cell supernatants (29) were analyzed for binding to GPNMB by BiaCore® 2000 biosensor equipped with a research-grade CM5 sensor chip. A 1:25 dilution of cell supernatant was passed over a protein A surface for 5 min followed by washing the surface for 10 mins. Subsequently, GPNMB was injected for 90 sec. over the surface at a concentration of 880 nM followed by dissociation. Double-referenced binding data were obtained by subtracting the signal from a control flow cell and subtracting the baseline drift of a buffer injected just prior to the antigen injection. GPNMB binding data for each mAb was normalized for the amount of mAb captured on each surface. Normalized, drift corrected responses were also measured. The sensorgrams were fit to a simple 1:1 kinetic model. The results are shown in Table 7. Sixteen of the cell supernatants contained mAb that significantly bound to GPNMB and three Mabs, 15.1, 15.2, and 15.3 showed strong binding to GPNMB.

Table 7

Sample	Kd (nM)	ka (M ⁻¹ s ⁻¹)	kd (s ⁻¹)	Expression Level
15.1	52	16524	8.55E-04	medium
15.3	59	13417	7.97E-04	medium
15.2	61	12635	7.70E-04	high
2.2	96	9257	8.90E-04	medium
10.2	118	3955	4.66E-04	low
7.3	121	9648	1.17E-03	medium
7.1	122	11842	1.44E-03	medium
7.2	141	9356	1.32E-03	high
10.3	147	3626	5.32E-04	low
10.1	209	4235	8.85E-04	low
8.2	242	7555	1.83E-03	medium
8.3	264	6551	1.73E-03	low
8.1	329	6830	2.25E-03	medium
12.3	407	1549	6.31E-04	medium
12.2	435	1280	5.57E-04	medium
12.1	630	1587	1.00E-03	low
1.1	>1000	<1500	nd	high

1.2	>1000	<1500	nd	high
1.3	>1000	<1500	nd	medium
2.1	>1000	<1500	nd	medium
5.1	>1000	<1500	nd	medium
5.2	>1000	<1500	nd	medium
5.3	>1000	<1500	nd	medium
9.1	>1000	<1500	nd	medium
9.2	>1000	<1500	nd	low
9.3	>1000	<1500	nd	low
11.1	>1000	<1500	nd	low
11.2	>1000	<1500	nd	low
11.3	>1000	<1500	nd	low

Example 4: Binning of Antibodies

Certain antibodies, described herein were binned in accordance with the protocol described in U.S. Patent Application Publication No.20030157730. MxhIgG conjugated beads are prepared for coupling to primary antibody. The volume of supernatant needed is calculated using the following formula: $(n+10) \times 50\mu\text{L}$ (where n = total number of samples on plate). Where the concentration is known, $0.5\mu\text{g/mL}$ is used. Bead stock is gently vortexed, then diluted in supernatant to a concentration of 2500 of each bead per well or $0.5 \times 10^5/\text{mL}$ and incubated on a shaker in the dark at RT overnight, or 2 hours if at a known concentration of $0.5\mu\text{g/mL}$. Following aspiration, $50\mu\text{L}$ of each bead is added to each well of filter plate, then washed once by adding $100\mu\text{L/well}$ wash buffer and aspirating. Antigen and controls are added to filter plate $50\mu\text{L/well}$ then covered and allowed to incubate in the dark for 1 hour on shaker. Following a wash step, a secondary unknown antibody is added at $50\mu\text{L/well}$ using the same dilution (or concentration if known) as is used for the primary antibody. The plates are then incubated in the dark for 2 hours at RT on shaker followed by a wash step. Next, $50\mu\text{L/well}$ biotinylated mxhIgG diluted 1:500 is added and allowed to incubate in the dark for 1 hour on shaker at RT. Following a wash step, $50\mu\text{L/well}$ Streptavidin-PE is added at 1:1000 and allowed to incubate in the dark for 15 minutes on shaker at RT. Following a wash step, each well is resuspended in $80\mu\text{L}$ blocking buffer and read using Luminex. Results show that the monoclonal antibodies belong to distinct bins. Competitive binding by antibodies from different bins supports antibody specificity for similar or adjacent epitopes. Non competitive binding supports antibody specificity for unique epitopes.

Three bins were created to further test the binding of six anti-GPNMB antibodies. Bin 1 included GPNMB antibodies (1.2.1), (1.10.1), and (2.22.1). Bin 2 included GPNMB antibodies (2.3.1) and (1.15.1), and Bin 3 included GPNMB antibody (2.10.1). The results of the binning assays are provided below in Tables 8 and 9.

5

Table 8

	BB	1.1	1.2	1.3	1.5	1.7	1.8	1.9	1.11	1.12	1.13	1.15	xV5
BB	0	16	58	24	6	25	14	9	8	9	7	15	32
1.1	-16	0	57	16	-29	34	9	-35	-9	-7	-24	35	28
1.2	-42	-16	0	-60	-89	-49	-81	-75	-73	-65	-81	-43	45
1.3	-11	-33	8	0	-75	-40	-49	171	-29	-33	-67	-73	-15
1.5	25	35	64	60	0	20	10	24	17	27	12	-8	61
1.7	-1	76	65	20	-8	0	-8	4	4	6	-3	-3	95
1.8	-7	29	45	35	-3	-7	0	4	-1	0	-6	3	52
1.9	-5	18	47	-7	-10	3	4	0	4	5	-5	-1	17
1.11	18	40	60	29	-11	1	15	16	0	8	5	-23	48
1.12	-10	26	43	27	-5	3	-12	-4	-12	0	-9	-13	57
1.13	1	30	40	27	2	9	2	10	11	17	0	-13	59
1.15	-19	91	79	71	15	21	8	12	10	15	13	0	89
xV5	41	134	239	46	5	443	230	-1	70	257	24	535	0
	I	II	III	IV	V	VI	VII	VIII		IX			
	1.1	1.2	1.3	1.5	1.7	1.8	1.9	1.15		xV5			
				1.13		1.11							
						1.12							

Table 9

	1.1	1.2	1.3	1.5	1.7	1.8	1.9	1.11	1.12	1.13	1.15	xV5	BB
1.1	0	72	39	-36	49	8	-14	-3	18	-14	35	28	-2
1.2	10	0	-60	-103	-46	-64	-76	-71	-69	-83	-74	44	-46
1.3	-49	-9	0	-111	-88	-78	281	-66	-57	-93	-115	-89	-33
1.5	61	106	77	0	13	28	17	20	40	2	-3	87	19
1.7	94	77	51	-25	0	-9	-3	12	4	-4	-17	96	17
1.8	42	71	74	-24	2	0	-9	1	-1	-12	-5	61	4
1.9	14	74	28	-24	6	4	0	3	5	-13	8	16	-17
1.11	59	66	77	-20	3	-5	13	0	11	-9	-5	92	21
1.12	84	67	61	-36	-12	-8	-6	-4	0	-16	-34	95	12
1.13	74	93	49	-12	22	12	23	21	19	0	20	98	55
1.15	127	90	51	-9	17	12	19	19	21	5	0	125	59
xV5	189	330	22	14	611	376	-17	113	445	44	750	0	100
BB	25	73	65	3	34	23	14	19	22	13	39	44	0
				I	II	III	IV	V	VI	VII		VIII	
		Cut-off = 100		1.1	1.2	1.3	1.5	1.7	1.9	1.15		xV5	
								1.8					
								1.11					
								1.12					
								1.13					
				I	II	III	IV	V	VI	VII	VIII		IX
		Cut-off = 90		1.1	1.2	1.3	1.5	1.7	1.8	1.9	1.15		xV5
							1.13		1.11				
								1.12					

Example 5: GPNMB Immunohistochemistry (IHC) Analysis

- 5 Anti-GPNMB monoclonal antibodies were evaluated for reactivity with frozen and fixed tissue specimens. Tissue sections (5 μ m) were cut from formalin fixed and paraffin embedded tissue samples and were rehydrated through incubations in xylene and a graded ethanol series terminating in PBS. Endogenous peroxidase activity was quenched in a 3% solution of hydrogen peroxide in methanol.
- 10 Tissue sections were blocked in blocking buffer (5% BSA (Sigma), 1% goat serum (Jackson Immunolabs, West Grove, PA) in PBS) for 1 hour. Primary and secondary antibodies were precomplexed in 5% BSA and 1% goat serum in PBS for 1 hour at 37° C at a molar ratio of approximately 10:1 of anti-GPNMB or control IgG to secondary biotinylated goat anti-human IgG (Jackson Immunolabs). Complexes were blocked with a
- 15 1:2000 dilution of human serum and incubated again for 1 hour at 37° C. Tissue sections were incubated with anti-GPNMB antibody or isotype control antibody complexes diluted

in blocking buffer for 1 hour. Sections were washed in 3 changes of PBS for 5 to 10 minutes each and incubated with a 1:200 dilution of streptavidin conjugated horseradish peroxidase (Jackson Immunolabs) in blocking buffer for 30 minutes and then washed as before. Antibody was detected using DAB reagent (Vector labs). Sections were
 5 counterstained in hematoxylin (Fisher Scientific) and dehydrated through alcohol and xylene and coverslipped with permount (Fisher Scientific).

Anti-GPNMB Mabs 2.22.1 and 2.22.2 were used to stain normal and tumor human tissue microarrays (IMPATH, Los Angeles, CA). Positive staining was seen in lung, ovarian, renal, esophagus, and head & neck carcinomas, squamous cell carcinoma,
 10 melanomas and normal skin specimens. Melanoma and lung carcinomas showed the highest staining intensities with subcellular staining located in the membrane and cytoplasm. Anti-GPNMB Mab 2.10.2 also stained primary melanoma.

Anti-GPNMB antibody staining of melanoma tissue microarray showed a large proportion of melanoma cases to be positively stained as shown in Tables 10 and 11.

15

Table 10: anti-GPNMB Mab Melanoma Staining Intensity

Staining Intensity*	# of Samples	%
0	10	17
1	1	2
1-2	2	3
1-3	11	19
2	9	15
2-3	17	29
3	9	15
Total	n=59	100

On a scale of 0 (no staining) to 3 (strong staining)

Table 11: anti-GPNMB Mab Staining Frequency

%Tumor Reactivity*	# Samples	%
0-24	18	31
25-49	6	10
50-74	7	12
75-100	28	47
	n=59	100%

* % tumor cells exhibiting positive staining

Anti-GPNMB antibody stained 10 of 14 lung squamous cell carcinoma (SCC) samples in a general oncology tissue microarray and 24 of 60 in a SCC specific array were positive.

5 **Example 6: FACS analysis of anti-GPNMB antibody binding to Melanoma cell lines**

The specificity of anti-GPNMB antibodies to cell membrane-bound GPNMB protein expressed by melanoma cancer cell line, UACC-62 was analyzed by FACS analysis. A renal cancer cell line, TK10, which does not express GPNMB antigen was used as a negative control. Isotype matched antibody pK16.3 was used as a negative control. Cells
 10 were washed twice with PBS (Ca and Mg free), incubated with Versene at 37°C until cells detached, counted and aliquoted at 1 million cells per assay tube. Cells were then washed twice and resuspended in ice-cold FACS buffer (0.01M HEPES, 0.15M NaCl, 0.1% NaN₃ and 4% FBS). Primary antibody at 1 µg/mL was added to the cells. Cells were incubated on ice for 30 min, washed 2-3 times and resuspended in 1 mL of ice-cold FACS buffer. R-PE-
 15 conjugated goat anti-human antibody (Jackson ImmunoResearch Laboratory) at 1:100 dilution was added and cells were incubated on ice for 30 min. After washing 3 times with 1 mL of ice-cold FACS buffer, cells were fixed with 0.5-1 mL of 1 % formaldehyde in PBS and analyzed by flow cytometry.

Results expressed as Geo Mean Ratios are summarized in Table 12 and show
 20 UACC-62 cells but not TK10 cells highly express CR011 protein on the cell surface which was detected by 2.10.2; 2.22.1 and 1.15.1 antibodies.

Table 12: Geo Mean Ratio of anti-GPNMB Staining (relative to pK16)

A.	Antibody	B.	UACC-62 Cells	C.	TK10 Cells
D.	CR011.2.10.2	E.	2.60	F.	1.10
G.	CR011.2.22.1	H.	4.46	I.	1.10
J.	CR011.1.2.2	K.	1.24	L.	0.98
M.	CR011.1.15.1	N.	7.89	O.	0.96
P.	CR011.2.6.2	Q.	1.90	R.	1.70

To examine the relative GPNMB antigen expression among melanoma cell lines, MAb 1.15.1 antibody was used to survey a panel of 15 melanoma cell lines by FACS analysis. As shown in Table 13, 80% (12/15) of cell lines showed GPNMB antigen expression. Cell line SK-Mel-2 demonstrated the highest Geo Mean ratio among the cell lines tested.

Table 13: Geo Mean Ratio of anti GPNMB Staining of Melanoma Cell Lines

Cell Line	Geo Mean Ratio (relative to isotype)
SK-Mel 2	16.5
M14	16.1
MEWO	14.1
WM-266-4	13.6
HEMNLP	10.2
G361	8
HT144	7.4
UACC-257	7
RPMI-7951	6
SK-Mel 5	5.7
UACC-62	5.5
A2058	4.1
SK-Mel 24	1.9
WM115	1.3
LOXIMVI	1

10 Example 7: FACS analysis of anti-GPNMB MAb binding to Lymphoma and Leukemia

To determine the relative expression of GPNMB on the surface of hematopoietic malignant cells, cell lines derived from various lymphomas and leukemias were incubated with anti-GPNMB antibody and analyzed by FACS. Lymphoma or leukemia derived cells were washed twice with ice-cold FACS buffer and resuspended at 1 million cells per assay tube. MAb 1.15.1 antibody at 1 µg/mL was added to cells and cells were incubated on ice

for 30 min. Cells were then washed 2-3 times and resuspended in 1 mL of ice-cold FACS buffer. R-PE-conjugated goat anti-human antibody at 1:100 dilution was added and cells were incubated on ice for 30 min. Cells were washed 3 times with 1 mL of ice-cold FACS buffer, fixed with 0.5-1 mL of 1 % formaldehyde in PBS and analyzed by Flow Cytometry.

- 5 Approximately half of the cell lines examined, which were derived from both myeloid and lymphoid lineages, showed GPNMB cell surface expression (Table 14). Cell line U937 demonstrated the highest Geo Mean ratio among the cell lines tested.

Table 14: Geo Mean Ratio of anti-GPNMB Staining of Lymphoma and Leukemia Cells

Cell line	Geo Mean Ratio
U937 (histiocytic lymphoma, monocytic)	17.3
Jurkat (acute T-cell leukemia)	14.7
SR (anaplastic large T cell lymphoma, ALCL)	7.1
KG-1 (acute myelogenous leukemia)	6.9
MOLT-4 (acute T cell lymphoblastic leukemia)	6.2
THP-1 (acute monocytic leukemia)	6.1
MV4-11 (myelomonocytic leukemia)	1.9
AML-193 (acute monocytic leukemia)	1.8
HUT-78 (T cell lymphoma)	1.5
CCRF-CEM (acute T cell lymphoblastic leukemia)	10.9
Karpas 299 (ALCL)	10.7
SU-DHL-1 (ALCL)	4.8
SU-DHL-4 (B cell lymphoma)	1.8
ML-2 (acute myelomonocytic leukemia)	2.1
HH (cutaneous T-cell leukemia)	1
SUP-M2 (ALCL)	4.8
PL-21 (acute myeloid leukemia)	12
DEL (ALCL)	7.9
SIG-M5 (acute monocytic leukemia)	2.9
K562 (Chronic myelogenous leukemia)	2.8
KG1a (acute myelogenous leukemia)	2.7
HL-60 (acute promyelocytic leukemia)	2.3
WSU-NH2 (B cell lymphoma)	1
EOL-1 (acute myeloid leukemia)	1
HUT-102 (T cell lymphoma)	1

10 **Example 8: Detection of GPNMB protein by IP and Western Blot Analysis**

- Cells were washed twice with PBS (Ca and Mg free), incubated with Versene at 37°C until cells detached, counted, collected and lysed in lysis buffer (0.15M NaCl, 0.02M Tris HCl, 10% glycerol, 1% NP-40, 0.01M EDTA and protease inhibitors containing pancreas extract, pronase, thermolysin, chymotrypsin and papain (Roche, Germany) for 30 min on ice. Supernatants were collected and protein concentrations were determined by
- 15 BCA protein assay kit (Pierce, USA). Primary antibody was added to the cell lysates and

incubated on ice for 3 hr followed by addition of Protein-G agarose (Amersham, USA) for 2 hr. Immunoprecipitated proteins were washed, boiled in sample buffer and resolved by 4-20% gels. For immunoblotting, proteins were transferred to PVDF membranes (Invitrogen, USA) and probed with anti-GPNMB antibody (0.5 µg/mL) followed by HRP-conjugated
5 goat anti-human antibody (Jackson ImmunoResearch Laboratory) at 1:4000 dilution. The immunocomplexes were detected with ECL Western blotting detection reagents (Amersham, USA).

Western blot analysis showed anti-GPNMB antibodies immunoprecipitated GPNMB protein expressed in cell lysates of UACC-62, SK-Mel5 and SK-Mel2 cell lines.
10 The results are in concurrence with the cell surface expression determined by FACS analysis.

Example 9: anti GPNMB Antibody mediated indirect cell killing

UACC-62, a GPNMB antigen expressing cell line, and TK10, a non-expressing cell
15 line were plated onto flat bottom 96-well tissue culture plates (Becton Dickinson, Franklin Lakes, NJ, USA) at a density of 3000 cells per well. Once the cells reached ~25% confluency, 100 ng/well of secondary antibody-toxin conjugate (goat anti-human IgG-saporin; Advanced Targeting Systems, San Diego, USA, HUM-ZAP; cat. # IT-22) was added. Anti-GPNMB MAbs 2.10.2, 2.22.1, 1.15.1 or isotype control mAb (pK16.3) were
20 added to each well at a final concentration of 10 or 50 ng/mL. An anti-EGFR monoclonal antibody (MS-269-PABX, NeoMarkers, Fremont, CA, USA) was used as a positive primary antibody control. Chemotherapy reagent 5-FU at 600 µM was used as a positive reagent control. On day 5, the cells were trypsinized, transferred to 6-well tissue culture plates and incubated at 37 °C. Plates were examined daily and between 8-10 days, all plates were
25 Giemsa stained and colonies were counted.

The percent viability of GPNMB positive UACC-62 after treatment is shown in Figure 2. Chemotherapy reagent 5-FU induced a complete killing whereas addition of saporin toxin-conjugated secondary antibody alone or in combination with isotype control pK16.3 antibody had no effect on cell growth for both cell lines. Both UACC-62 and TK10
30 cell lines express EGFR protein and addition of EGFR specific antibody at 50 ng/mL and secondary antibody toxin conjugate resulted in a complete killing of UACC-62 and TK10 cells. At the same dose, all three GPNMB specific antibodies, 2.10.2, 2.22.1 and 1.15.1

induced over 70% killing of UACC-62 cells. Anti-GPNMB antibodies 2.10.2 and 2.22.1 induced less than 5% and 1.15.1 less than 24% cell death in GPNMB negative TK10 cells.

Example 10: Cell killing by Auristatin-E (AE) Conjugated anti-GPNMB Antibodies

5 UACC-62 and TK10 cells were plated onto flat bottom 96-well tissue culture plates (Becton Dickinson, Franklin Lakes, NJ, USA). On day 2 or cells reach ~25% confluency, various concentrations (1 to 1000 ng/mL) of unconjugated and Auristatin E-conjugated antibodies (Seattle Genetics, Bothell, WA, USA), including isotype control, EGFR (NeoMarkers MS-269-PABX, Fremont, CA, USA), 2.22.1 or 2.10.2, were added to cells.
10 MAb 2.3.1 was chosen for the isotype control in this study because it does not bind to GPNMB expressing cells as demonstrated by FACS analysis. A monoclonal antibody generated against the EGF receptor was used to demonstrate specific killing mediated by AE-conjugated antibody. On day 5, the cells were trypsinized, transferred to 6-well tissue culture plates and incubated at 37°C. Plates were examined daily. On days 8-10, all plates
15 were Giemsa stained and colonies on the plates were counted.

The percent viability in GPNMB positive UACC-62 cells and negative TK10 cells is presented in Figures 4 and 5, respectively. The results indicate that unconjugated and AE-conjugated 2.6.2 immunoconjugate had no effect on growth of both UACC-62 and TK10 cells. However, both UACC-62 and TK10 cell lines were susceptible to AE-EGFR
20 immunoconjugate mediated cell killing in a dose-dependent fashion with over 95 % cell death at 1000 ng/mL. At the same dose, both 2.22.1-AE and 2.10.2-AE immunoconjugates induced approximately 75 % cell death of UACC-62 cells when compared to the isotype control. The cell killing response was dose dependent. GPNMB negative TK10 cell survival was not affected by 2.22.1-AE nor 2.10.2-AE immunoconjugates at the same dose
25 range. These results demonstrate the specific and cytotoxic effects of AE conjugated anti-GPNMB antibodies on antigen expressing cells.

Example 11: Melanoma cells susceptible to MAb1.15.1-AE immunoconjugate killing

Melanoma cell lines were plated onto flat bottom 96-well tissue culture plates
30 (Becton Dickinson, Franklin Lakes, NJ, USA). On day 2 or when cells reach ~25% confluency, various concentrations of unconjugated and Auristatin E-conjugated 1.15.1 were added to cells. MAb 2.6.2-AE was also used as a conjugated isotype control in this study. On day 5, the cells were trypsinized, transferred to 6-well tissue culture plates and

incubated at 37 °C. Plates were examined daily. On days 8-10, all plates were Giemsa stained and colonies on the plates were counted.

The IC₅₀ of 1.15.1-AE mediated killing on GPNMB positive and negative cells are presented in Table 15. Unconjugated 1.15.1 and AE-conjugated 2.6.2 had no effect on growth of all the melanoma cell lines tested. However, cell lines SK-Mel2, WM-266-4, G361, UACC-257, UACC-62, RPMI-7951 and SK-Mel5 were susceptible to 1.15.1-AE mediated killing in a dose-dependent fashion. SK-Mel2 demonstrated the lowest IC₅₀ in this study (Table 15). These results show the specific and cytotoxic effects of AE conjugated 1.15.1 on most of GPNMB expressing melanoma cells.

Table 15: Geo Mean Ratios and IC₅₀ Values of 1.15.1-AE Killing of Melanoma Cells

Melanoma Cell Line	Geo Mean Ratio (relative to isotype)	Clonogenic Assay with 1.15.1-AE IC50 in ng/mL (pM)
SK-Mel 2	16.5	111 (750)
M14	16.1	Inconclusive
MEWO	14.1	Inconclusive
WM-266-4	13.6	345 (2300)
HEMNLP	10.2	Inconclusive
G361	8	1053 (6500)
HT144	7.4	Inconclusive
UACC-257	7	825 (5500)
RPMI-7951	6	972 (6000)
SK-Mel 5	5.7	237 (1600)
UACC-62	5.5	697 (4300)
A2058	4.1	No effect
SK-Mel 24	1.9	No data
WM115	1.3	No data
LOXIMVI	1	No effect

Example 12: MAb 1.15.1-AE killing of Lymphoma and Leukemia cell lines

Lymphoma or leukemia cell lines were mixed with methylcellulose base media (R&D Systems, USA) and in various concentrations of unconjugated and Auristatin E-conjugated 1.15.1 antibody before plating onto 6-well tissue culture plates (Becton Dickinson, Franklin Lakes, NJ, USA). MAb 2.6.2-AE was also included as a conjugated isotype control in this study because it does not bind to GPNMB expressing cells. Plates were incubated at 37 °C and examined daily. On days 14-18, colonies on the plates were counted.

The IC₅₀ of 1.15.1-AE induced cell killing on antigen expressing cells is presented in Table 16. Unconjugated 1.15.1 and AE-conjugated 2.6.2 immunoconjugate had no effect on growth of all antigen positive hematopoietic cell lines. However, as presented in Table 16, cell lines U937, SR and THP-1 derived from either myeloid or lymphoid lineage were susceptible to 1.15.1-AE mediated killing in a dose-dependent manner with IC₅₀ values ranging from 207 ng/mL (1.4 nM) to 340 ng/mL (2.4 nM). These results show the specific and cytotoxic effects of 1.15.1-AE immunoconjugate on GPNMB antigen expressing hematopoietic malignant cell lines.

Table 16: Geo Mean Ratios and IC₅₀ Values of 1.15.1-AE Killing of Lymphoma and Leukemia Cells

			Clonogenic Assay with 1.15.1-AE	
Cell line		Geo Mean Ratio	IC50 in ng/mL	(pM)
U937	(histiocytic lymphoma, monocytic)	17.3	340	(2400)
Jurkat	(acute T-cell leukemia)	14.7	No effect (repeating)	
SR	(anaplastic large T cell lymphoma, ALCL)	7.1	296	(2000)
KG-1	(acute myelogenous leukemia)	6.9	No growth	
MOLT-4	(acute T cell lymphoblastic leukemia)	6.2	No effect (repeating)	
THP-1	(acute monocytic leukemia)	6.1	207	(1400)
MV4-11	(myelomonocytic leukemia)	1.9	ND	
AML-193	(acute monocytic leukemia)	1.8	ND	
HUT-78	(T cell lymphoma)	1.5	ND	
CCRF-CEM (acute T cell lymphoblastic leukemia)		10.9	No growth	
Karpas 299	(ALCL)	10.7	Inconclusive	
SU-DHL-1	(ALCL)	4.8	No effect	
SU-DHL-4	(B cell lymphoma)	1.8	ND	
ML-2	(acute myelomonocytic leukemia)	2.1	ND	
HH	(cutaneous T-cell leukemia)	1	ND	
SUP-M2	(ALCL)	4.8	No growth	
PL-21	(acute myeloid leukemia)	12	No effect	
DEL	(ALCL)	7.9	No effect	
SIG-M5	(acute monocytic leukemia)	2.9	ND	
K562	(Chronic myelogenous leukemia)	2.8	ND	
KG1a	(acute myelogenous leukemia)	2.7	ND	
HL-60	(acute promyelocytic leukemia)	2.3	ND	
WSU-NH2	(B cell lymphoma)	1	ND	
EOL-1	(acute myeloid leukemia)	1	ND	
HUT-102	(T cell lymphoma)	1	ND	
* ND: Not done				

Example 13: CR011-vcMMAE Inhibits the Growth of Human SK-MEL-2 Melanoma Xenografts Leading to Complete Regression of Established Melanoma Tumors in Athymic Mice (Study N-386)

5 Study N-386 was performed to assess the potency and therapeutic efficacy of the antibody-drug conjugate, CR011-vcMMAE, against the established human SK-MEL-2 melanoma xenograft in athymic mice.

Materials and Methods:

10 **Test Animals:** Five- to 6-week old athymic mice (CD-1 *nu/nu* females), used for human tumor xenografts, were obtained from Harlan Laboratories (Indianapolis, IN). Animals were housed in specific pathogen-free conditions, according to the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). Test animals were provided pelleted food and water *ad libitum*
15 and kept in a room with conditioned ventilation (HVAC), temperature ($22^{\circ} \pm 2^{\circ}\text{C}$), relative humidity ($55\% \pm 15\%$), and photoperiod (12 hr). All studies were carried out with approved institutional animal care and use protocols.

Human Melanoma Xenograft Models. The tumor inhibitory activity of the CR011-MMAE immunoconjugate was measured in an anti-tumor xenograft model using
20 athymic mice, according to published methods (see Geran *et al.*, Cancer Chemother. Rep. 3:1-104 (1972)). Briefly, test animals were implanted subcutaneously by trocar with small fragments of a human melanoma (60-125 mg) excised from athymic mouse tumor donors. When tumors became established (10-20 days), the animals were pair-matched into groups ($n=6$ mice/group), and treatment was administered by intravenous injection (tail vein).

25 The SK-MEL-2 human melanoma (ATCC #HTB-68) was derived from a metastatic site (skin of thigh) of a 60 year old Caucasian male with malignant melanoma, and the SK-MEL-5 human melanoma (ATCC #HTB-70) was derived from a metastatic site (axillary lymph node) of a 24 year old Caucasian female with malignant melanoma (see Fogh *et al.*, J. Natl. Cancer Inst. 59: 221-226 (1977)). Both cell lines were obtained from the American
30 Type Culture Collection.

The effects of treatment were monitored by repetitive tumor measurements across 2 diameters with Vernier calipers; tumor size (in mg) was calculated using a standard formula, $(W^2 \times L)/2$, assuming a specific gravity of 1.0. Tumor size and body weights were assessed twice weekly. Mice were examined daily, however, and moribund animals were
35 humanely euthanized if clinical indications of excessive pain or distress were noted (*i.e.*,

prostration, hunched posture, paralysis/paresis, distended abdomen, ulcerations, abscesses, seizures, and/or hemorrhages). Animals with tumors exceeding 2,000 mg were removed from the study and euthanized humanely.

Xenograft studies in the athymic mouse have been shown to effectively demonstrate anti-tumor effects for a variety of agents which have been shown subsequently to have activity against clinical cancer (Johnson *et al.*, *Br J Cancer* 84:1424-1431 (2001)).

Results:

Anti-Tumor Effects *In Vivo* vs. SK-MEL-2 Melanoma. Based on the potency and cytotoxicity of CR011-vcMMAE against GPNMB-expressing cells *in vitro*, the anti-tumor effects were examined *in vivo*.

The effects of intravenous CR011-vcMMAE treatment on the growth of subcutaneous human SK-MEL-2 melanoma are shown in Figure 1. After SK-MEL-2 tumor fragments were implanted and tumors became established (day 17, 61 mg), treatment commenced with intravenous administration of: CR011-vcMMAE (0.625 – 20 mg/kg i.v., every 4 days for a total of 4 treatments (*i.e.*, q4d X4); saline and phosphate-buffered saline controls (i.v., q4d X4); and two known anti-tumor reference agents, vinblastine sulfate (i.v., 1.7 mg/kg, q4d X4) and paclitaxel (i.v., 24 mg/kg, q2d X4). The reference agents were administered at the maximum tolerated dose (MTD) determined in prior studies.

Tumors in animals treated with saline or PBS grew progressively until the tumor mass reached 2,000 mg at which time the animals were removed from the study and euthanized humanely. SK-MEL-2 tumors have a high “take” rate in immunocompromised hosts (97 %) and a low rate of spontaneous regression (3 %) (Dykes *et al.*, Development of human tumor xenograft models for *in vivo* evaluation of new antitumor drugs, in Immunodeficient mice in Oncology, vol. 42 (Fiebig HH and Berger DPe eds) pp 1-22, Contrib. Oncol. Basel, Karger (1992)).

Vinblastine produced a very slight, but not significant, anti-tumor effect ($P \leq 0.20$); in this and other tumor models (*e.g.*, SK-MEL-5) vinblastine produces noticeable tumor growth inhibition, but which is only occasionally significant. Paclitaxel, however, showed significant tumor growth inhibition and tumor stasis (*i.e.*, 100% growth inhibition) for approximately 2 weeks after treatment commenced ($P \leq 0.0077$).

The anti-tumor effects of CR011-vcMMAE administered i.v. to SK-MEL-2-bearing mice were remarkable. At 20, 10, 5 or 2.5 mg/kg tumors rapidly diminished in size for the majority of the test animals; significant treatment effects were noted as early as 4 days after

treatment commenced ($P \leq 0.014$). Tumors that regressed completely did not re-grow during the observation period (> 200 days).

The animals in this study showed no abnormal treatment effects on gross examination. Twice weekly body weight determinations showed no observable or statistically significant effects of treatment with CR011-vcMMAE on body weight or weight gain.

Conclusions:

CR011-vcMMAE produces substantial, dose-dependent and reproducible anti-tumor effects that begin as tumor growth inhibition but soon lead to complete regression of established human melanoma xenografts; the regressions are long-lived and re-growth of tumors after successful therapy has not been observed.

Example 14: Sequencing of Antibodies and their Corresponding DNA

Sequences of human GPNMB mAbs-derived heavy and kappa chain transcripts from hybridomas were obtained by direct sequencing of PCR products generated from poly(A⁺) RNA. PCR products were also cloned into pCRII using a TA cloning kit (Invitrogen) and both strands were sequenced using Prism dye-terminator sequencing kits and an ABI 377 sequencing instrument. Each PCR reaction used a mixture of 5' sense primers which are provided in Table 17 below.

Table 17: Primers Used

VH	cacc ATG GAC TGG(C) ACC TGG AGG ATC	SEQ ID NO: 290
VH	cacc ATG GAC TGG ACC TGG AGA(C) ATC	SEQ ID NO: 291
VH	cacc ATG GAC TGG ACC TGG AGG GTC	SEQ ID NO: 292
VH	cacc ATG GAC TGG ATT TGG AGG ATC	SEQ ID NO: 293
VH	cacc ATG GAC ACA CTT TGC TC(A)C AC	SEQ ID NO: 294
VH	cacc ATG GAA(G) TTG GGG CTG AGC TGG	SEQ ID NO: 295
VH	cacc ATG GAG TTG(T) GGA CTG AGC TGG	SEQ ID NO: 296
VH	cacc ATG GAG TTT GGG CTG(T) AGC TGG	SEQ ID NO: 297
VH	cacc ATG GAA CTG GGG CTC CGC TGG	SEQ ID NO: 298
VH	cacc ATG GAG TTG GGG CTG TGC TGG	SEQ ID NO: 299
VH	cacc ATG GAG TTT TGG CTG AGC TGG	SEQ ID NO: 300
VH	cacc ATG ACG GAG TTT GGG CTG AGC	SEQ ID NO: 301
VH	cacc ATG AAA(G) CAC CTG TGG TTC TTC	SEQ ID NO: 302
VH	cacc ATG AAA CAT CTG TGG TTC TTC	SEQ ID NO: 303
VH	cacc ATG GGG TCA ACC GCC ATC CTC	SEQ ID NO: 304
VH	cacc ATG TCT GTC TCC TTC CTC ATC TTC	SEQ ID NO: 305
VK	ATG GGG TCC CAG GTT CAC CTC	SEQ ID NO: 306
VK	ATG TTG CCA TCA CAA CTC ATT G	SEQ ID NO: 307

All sequences were analyzed by alignments to the "V BASE sequence directory" (Tomlinson *et al.*, MRC Centre for Protein Engineering, Cambridge, UK) using MACVECTOR® and GENEWORKS™ software programs.

Example 15: Structural Analysis of Anti-GPNMB Antibodies

- 5 The variable heavy chains and the variable light chains for the antibodies shown in Table 17 were sequenced to determine their DNA and protein sequences.

Antibody -1.10.2

Heavy chain variable region

Nucleotide sequence

- 10 5' AGGTGCAGCTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCACTGTCTCTGGTGACTCCATCAGTAATTACTACTGGAGCTGGATCCGGCAGCCCCAGGGAAGGGACTGGAGTGGATTGGGTATTTCTATTACAGTGGGAGCACCACTACAACCCCTCCCTCAAGAGTCGAGTCACCATATCAGTAGACACGTC
CAAGAACCAGTTCTCCCTGAAACTGAGCTCTGTGACCGCTGCGGACACGGCCGTGTATTACTGTGCGAGAGATA
GGGGCTGGGCTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCC 3' (SEQ ID NO:1)

Amino acid sequence

- 15 5' QVQLQESGPGLVKPSSETLSLTCTVS GDSISNYYWS WIRQPPGKGLEWIG YFYSGSTNYPNPSLKS
RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR DRGWADY WGQGLTVTVSSA 3' (SEQ ID NO:2)

TABLE 18. 1.10.2 Heavy chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID NO:
FR1	QVQLQESGPGLVKPSSETLSLTCTVS	1-25	SEQ ID NO:3
CDR1	GDSISNYYWS	26-35	SEQ ID NO:4
FR2	WIRQPPGKGLEWIG	36-49	SEQ ID NO:5
CDR2	YFYSGSTNYPNPSLKS	50-65	SEQ ID NO:6
FR3	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	66-97	SEQ ID NO:7
CDR3	DRGWADY	98-104	SEQ ID NO:8
FR4	WGQGLTVTVSSA	105-116	SEQ ID NO:9

- 20 *AA Residues of SEQ ID NO:2

Light chain variable region

Nucleotide sequence

- 25 5' GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGGGCCACCCTCTCCTGCAGAACCCAGTCAGAGTATTAGCAGCAGCTATTTAGCCTGGTACCAGCAGAAACCTGGCCA
GGTTCCCAGGCTCCTCATCTATGGTGCTTCCAGCAGGGCCACTGGCATCCCAGACAGTTTCAGTG
GCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTG
TATTATTGTCAGCAGTATGGTAGCTCGATCACCTTCGGCCAAGGGACACGACTGGAGATTAAACG
A 3' (SEQ ID NO:10)

Amino acid sequence

- 30 5' EIVLTQSPGTLSPGERATLSC RTSQSISSSYLA WYQQKPGQVPRLLIY GASSRAT
GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC QQYGSSIT FGQGTRLEIKR 3' (SEQ ID NO:11)

TABLE 19. 1.10.2 Light chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FR1	EIVLTQSPGTLSPGERATLSC	1-23	SEQ ID NO:12
CDR1	RTSQSISSSYLA	24-35	SEQ ID NO:13
FR2	WYQQKPGQVPRLLIY	36-50	SEQ ID NO:14
CDR2	GASSRAT	51-57	SEQ ID NO:15
FR3	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	58-89	SEQ ID NO:16
CDR3	QQYGSSIT	90-97	SEQ ID NO:17
FR4	FGQGTRLEIKR	98-108	SEQ ID NO:18

*AA Residues of SEQ ID NO:11

Antibody -1.15.1

Heavy chain variable region

5 Nucleotide sequence

5' CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTCACCTGCACTGTC
TCTGGTGGCTCCATCAGCAGTTTTAATTACTACTGGAGCTGGATCCGCCACCACCCAGGGAAGGGCCT
GGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTACTCCAACCCGTCCCTCAAGAGTCGAGTTACC
ATATCAGTAGACACGTCTAAGAACCAGTTCTCCCTGACGCTGAGCTCTGTGACTGCCGCGGACACGGCCG
TGTATTACTGTGCGAGAGGGTATAACTGGAACACTTTGACTACTGGGGCCAGGGAACCTGGTCACCGT
CTCCTCAGCC 3' (SEQ ID NO:19)

Amino acid sequence

5' QVQLQESGPGLVKPSQTLSTCTVSGGSISFNYYWSWIRHHPGKGLEWIGYIYYSGSTYSNPSLKSRTVIS
VDTSKNQFSLTLSSVTAADTAVYYCARGYNWNYFDYWQGTLVTVSSA 3' (SEQ ID NO:20)

TABLE 20. 1.15.1 Heavy chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FRI	QVQLQESGPGLVKPSQTLSTCTVSGGSIS	1-30	SEQ ID NO:21
CDR1	SFNYYWS	31-37	SEQ ID NO:22
FR2	WIRHHPGKGLEWIG	38-51	SEQ ID NO:23
CDR2	YIYYSGSTYSNPSLKS	52-67	SEQ ID NO:24
FR3	RVTISVDTSKNQFSLTLSSVTAADTAVYYCAR	68-99	SEQ ID NO:25
CDR3	GYNWNYFDY	100-108	SEQ ID NO:26
FR4	WGQGLVTVSSA	109-120	SEQ ID NO:27

*AA Residues of SEQ ID NO:20

Light chain variable region

20 Nucleotide sequence

5' GAAATAGTGATGACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGG
GCCAGTCAGAGTGTGACAACAACCTTAGTCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCT
CATCTATGGTGCATCCACCAGGGCCACTGGTATCCCAGCCAGGTTCACTGGCAGTGGGTCTGGGACAGAG
TTCACCTCTACCATCAGTAGTCTGCAGTCTGAAGATTTTGCAGTTTATTACTGTCTCAGCAGTATAATAACT
GGCCTCCGTGGACGTTCCGGCCAAGGGACCAAGGTGGAATCAAACGA 3' (SEQ ID NO:28)

Amino acid sequence

5' EIVMTQSPATLSVSPGERATLSCRASQSDNNLVWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFT
LTISLSQSEDFAVYYCQQYNNWPPWTFGQGTKVEIKR 3' (SEQ ID NO:29)

TABLE 21. 1.15.1 Light chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FRI	EIVMTQSPATLSVSPGERATLSC	1-23	SEQ ID NO:30
CDR1	RASQSDNNLV	24-34	SEQ ID NO:31
FR2	WYQQKPGQAPRLLIY	35-49	SEQ ID NO:32
CDR2	GASTRAT	50-56	SEQ ID NO:33
FR3	GIPARFSGSGSGTEFTLTISLSQSEDFAVYYC	57-88	SEQ ID NO:34
CDR3	QQYNNWPPWT	89-98	SEQ ID NO:35
FR4	FGQGTKVEIKR	99-109	SEQ ID NO:36

*AA Residues of SEQ ID NO:29

Antibody -1.2.2

35 Heavy chain variable region

Nucleotide sequence

5' ATCACCTTGAAGGAGTCTGGTCCTACGCTGGTGAAACCCACACAGACCCTCAGCTGACC
 TGCACCTTCTCTGGGTCTCACTCAGCGCTGGTGGAGTGGGTGTGGGCTGGATCCGTCAG
 CCCCCAGGAAAGGCCCTGGAGTGGCTTGCACTCATTTATTGGAATGATGATAAGCGCTAC
 AGCCCATCTCTGAGGAGCAGGCTCACCATCACCAGGACACCTCCAAAAACCAGGTGGTC
 5 CTTACAATTACCAACATGGACCCTGTGGACACAGCCACATATTATTGTGCACACAGTCAC
 TATGATTACGATTGGGGAGTTACTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTC
 TCCTCAGCC 3' (SEQ ID NO:37)

Amino acid sequence

10 5' ITLKESGPTLVKPTQTLTLTCTFS GFSL SAGGVGVG WIRQPPGKALEWLA LIYWND DKRY
 SPSLRS RLITIKDTSKNQVVLITINMDPVD TATYYCAH SHYDYDWGSYFDY WQGGTLVTVSSA 3'
 (SEQ ID NO:38)

TABLE 22. 1.2.2 Heavy chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FRI	ITLKESGPTLVKPTQTLTLTCTFS	1-24	SEQ ID NO:39
CDR1	GFSL SAGGVGVG	25-36	SEQ ID NO:40
FR2	WIRQPPGKALEWLA	37-50	SEQ ID NO:41
CDR2	LIYWND DKRYSPSLRS	51-66	SEQ ID NO:42
FR3	RLITIKDTSKNQVVLITINMDPVD TATYYCAH	67-98	SEQ ID NO:43
CDR3	SHYDYDWGSYFDY	99-111	SEQ ID NO:44
FR4	WQGGTLVTVSSA	112-123	SEQ ID NO:45

15 *AA Residues of SEQ ID NO:38

Light chain variable region

Nucleotide sequence

20 5' GATATTGTGATGACCCAGACTCCACTCTCCCTGCCCCGTACCCCTGGAGAGCCGGCCTCC
 ATCTCCTGCAGGTCTAGTCAGAGCCTCTTGGATAGTGATGATGGAACACCTATTGGAC
 TGGTACCTGCAGAAGCCAGGACAGTCTCCACAGCTCCTGATCTATACGCTTTCCTATCGG
 GCCTCTGGAGTCCCAGACAGGTTCACTGGCAGTGGGTGAGGCACTGATTTCACTGAAC
 ATCAGCAGGGTGGAGGCTGAGGATGTTGGAGTTTATTACTGCATGCAACGTATAGAGTTT
 CCTATCACCTTCGCCAAGGGACACGACTGGAGATTAAACGA 3' (SEQ ID NO:46)

25

Amino acid sequence

5' DIVMTQTPLSLPVTGPGEPA SISC RSSQSLLDSDDGNTYLD WYLQKPGQSPQLLIY TLSYRAS
 GVPDRFSGSGSGTDFTLNISRVEAEDVGVYYC MQRIEFPIT FGQGRLEIKR 3' (SEQ ID NO:47)

30 TABLE 23. 1.2.2 Light chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FRI	DIVMTQTPLSLPVTGPGEPA SISC	1-23	SEQ ID NO:48
CDR1	RSSQSLLDSDDGNTYLD	24-40	SEQ ID NO:49
FR2	WYLQKPGQSPQLLIY	41-55	SEQ ID NO:50
CDR2	TLSYRAS	56-62	SEQ ID NO:51
FR3	GVPDRFSGSGSGTDFTLNISRVEAEDVGVYYC	63-94	SEQ ID NO:52
CDR3	MQRIEFPIT	95-103	SEQ ID NO:53
FR4	FGQGRLEIKR	104-114	SEQ ID NO:54

*AA Residues of SEQ ID NO:47

Antibody -1.7.1

Heavy chain variable region

Nucleotide sequence

35 5' CAGGTGCAGCTGCAGGAGTGGGGCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
 ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGCTAATTACTACTGGACCTGGATCCGC

CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTAC
 TGCAACCCGTCCTCAAGAGTCGAGTTATCATATCAGTAGACACGCTCTAAGAACCAGTTC
 TCCCTGAAGCTGAGCTCTGTGACTGCCCGGGACACGGCCGTGTATTACTGTGCGAGAGGG
 TATAACTGGAACCTTTGACTACTGGGGCCAGGGAACCCCTGGTCACCGTCTCCTCAGCC 3' (SEQ ID
 NO:55)

Amino acid sequence

5' QVQLQESGPGLVKPSQTLSTCTVS GGSISSANYWT WIRQHPKGLEWIG YIYYSGSTY
 CNPSLKS RVIISVDTSKNQFSLKLSSVTAADTAVYYCAR GYNWNYFDY WGQGLTVTVSSA 3' (SEQ
 ID NO:56)

TABLE 24.1.7.1 Heavy chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FRI	QVQLQESGPGLVKPSQTLSTCTV	1-25	SEQ ID NO:57
CDR1	GGSISSANYWT	26-37	SEQ ID NO:58
FR2	WIRQHPKGLEWIG	38-51	SEQ ID NO:59
CDR2	YIYYSGSTYCNPSLKS	52-67	SEQ ID NO:60
FR3	RVIISVDTSKNQFSLKLSSVTAADTAVYYCAR	68-99	SEQ ID NO:61
CDR3	GYNWNYFDY	100-108	SEQ ID NO:62
FR4	WGQGLTVTVSSA	109-120	SEQ ID NO:63

*AA Residues of SEQ ID NO:56

Light chain variable region

Nucleotide sequence

5' GATATAGTGTGACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGG
 GCCAGTCAGAGTGTAGCAGCAACTTAGCCTGGTACCAGGAGAGACCTGGCCAGGCTCCCAGACTCCTCATCTA
 TGGTGCATCCACCAGGGCCACTGGTATCCAGCCAGGTTCAAGTGGCAGTGGGTCTGGGACAGAGTTCACTCTCA
 CCATCAGCAGCCTGCAGTCTGAAGATTTTCAGTTTATTACTGTCAGCAGTATAATAAGTGGCCTCCGTGGACG
 TTCGGCCAAGGGACCAAGGTGGAAATCGAACGAACT 3' (SEQ ID NO:64)

Amino acid sequence

5' DIVMTQSPATLSVSPGERATLSC RASQSVSSNLA WYQERPGQAPRLLIY GASTRAT
 GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC QQYNKWPPWT FGQGTKVEIER 3' (SEQ ID
 NO:65)

TABLE 25. 1.7.1 Light chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FRI	DIVMTQSPATLSVSPGERATLSC	1-23	SEQ ID NO:66
CDR1	RASQSVSSNLA	24-34	SEQ ID NO:67
FR2	WYQERPGQAPRLLIY	35-49	SEQ ID NO:68
CDR2	GASTRAT	50-56	SEQ ID NO:69
FR3	GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC	57-88	SEQ ID NO:70
CDR3	QQYNKWPPWT	89-98	SEQ ID NO:71
FR4	FGQGTKVEIER	99-109	SEQ ID NO:72

*AA Residues of SEQ ID NO:65

Antibody -2.10.2

Heavy chain variable region

Nucleotide sequence

5' CAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCTCCTGT
 GCAGCCTCTGGATTTCGCTTCAGTAGCTATGGCATGCACTGGGTCCGCCAGGCTCCAGGC
 AAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAATAATAAATACTATGCAGAC
 TCCGTGAAGGGCCGATTACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAA
 ATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGATCTAGTGGTT

CGGGGAATTAGGGGGTACTACTACTTCTGGTATGGACGTCTGGGGCCAAGGGACCACG
GTCACCGTCTCCTCAGCC 3' (SEQ ID NO:73)

Amino acid sequence

- 5' QLVESSGGVVQPGRSRLRLSCAAS GFAFSSYGMH WVRQAPGKGLEWVA VISYDGNKYYAD
SVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR DLVVRGIRGYYYFQMDV WGQGT
TVSSA 3' (SEQ ID NO:74)

TABLE 26. 2.10.2 Heavy chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FRI	QLVESSGGVVQPGRSRLRLSCAAS	1-23	SEQ ID NO:75
CDR1	GFAFSSYGMH	24-33	SEQ ID NO:76
FR2	WVRQAPGKGLEWVA	34-47	SEQ ID NO:77
CDR2	VISYDGNKYYADSVKG	48-64	SEQ ID NO:78
FR3	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	65-96	SEQ ID NO:79
CDR3	DLVVRGIRGYYYFQMDV	97-114	SEQ ID NO:80
FR4	WGQGT TVSSA	115-126	SEQ ID NO:81

- 10 *AA Residues of SEQ ID NO:74

Light chain variable region

Nucleotide sequence

- 5' GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCCGTACCCCTGGAGAGCCGGCTCC
ATCTCTGCAGGTCTAGTCAGAGCCTCCTGCATAGTAATGGATACAACATTTGGATTGG
15 TACCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTATTTGGGTCTAATCGGGCC
TCCGGGTCCCTGACAGGTTCACTGGCAGTGGATCAGGCACAGATTTTACTGAAAATC
AGCAGAGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAGGTCTACAACTCCG
ATCACCTTCGGCCAAGGGACACGACTGGAGATTAAACGA 3' (SEQ ID NO:82)

- 20

Amino acid sequence

5' DIVMTQSPLSLPVTGPGEASISC RSSQSLLSNGYNYLD WYLQKPGQSPQLLIY LGSNRAS
GVPDRFSGSGGTDFTLKISRVEADVGVYYC MQGLQTPIT FGQTRLEIKR 3' (SEQ ID NO:83)

- 25 TABLE 27. 2.10.2 Light chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FRI	DIVMTQSPLSLPVTGPGEASISC	1-23	SEQ ID NO:84
CDR1	RSSQSLLSNGYNYLD	24-39	SEQ ID NO:85
FR2	WYLQKPGQSPQLLIY	40-54	SEQ ID NO:86
CDR2	LGSNRAS	55-61	SEQ ID NO:87
FR3	GVPDRFSGSGGTDFTLKISRVEADVGVYYC	62-93	SEQ ID NO:88
CDR3	MQGLQTPIT	94-102	SEQ ID NO:89
FR4	FGQTRLEIKR	103-113	SEQ ID NO:90

*AA Residues of SEQ ID NO:83

Antibody – 2.15.1

Heavy chain variable region

- 30 Nucleotide sequence

- 5' CAGGTGCAGCTGGTGGAGTCTGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTC
TCCTGTGCAGCGTCTGGATTACCTTCAGTAACATATGGCATTCACTGGGTCCGCCAGGCT
CCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTTTGATGGACGTAATAAATACTAT
GCAGACTCCGTGAAGGGCCGATTACCATCTCCAGAGACAATTCCAAGAACACGCTGTAT
35 CTGCAAATGAACAGCCTGAGAGCCGAGGACGCGGCTGTGTATTACTGTGCGAGAGATCCC
TTTGACTATGGTGACTCCTTCTTGACTACTGGGGCCAGGGCACCTGGTCACCGTCTCC
TCAGCC 3' (SEQ ID NO:91)

Amino acid sequence

5' QVQLVESGGGVVQPGRSLRLSCAAS GFTFSNYGIH WVRQAPGKGLEWVA VIWFDGRNKYY
 ADSVKG RFTISRDN SKNTLYLQMNSLRAEDAAVYYCAR DPFYDGSFFDY WGQGLTVTVSSA 3'
 5 (SEQ ID NO:92)

TABLE 28. 2.15.1 Heavy chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FRI	QVQLVESGGGVVQPGRSLRLSCAAS	1-25	SEQ ID NO:93
CDR1	GFTFSNYGIH	26-35	SEQ ID NO:94
FR2	WVRQAPGKGLEWVA	36-49	SEQ ID NO:95
CDR2	VIWFDGRNKYYADSVKG	50-66	SEQ ID NO:96
FR3	RFTISRDN SKNTLYLQMNSLRAEDAAVYYCAR	67-98	SEQ ID NO:97
CDR3	DPFDYDGSFFDY	99-110	SEQ ID NO:98
FR4	WGQGLTVTVSSA	111-122	SEQ ID NO:99

*AA Residues of SEQ ID NO:92

10 **Light chain variable region****Nucleotide sequence**

55' CTGACTCAGTCTCCATCCTCCCTGTCTGCATCTGTAAGAGACAGAGTCACCATCACTTGC
 CGGGCGAGTCAGGACATTAGCAATTATTTAGCCTGGTATCAGCAGAAACCAGGAAAGTT
 CCTAATCTCCTGATCTATGCTGCATCCACTTTGCAATCAGGGGTCCCATCTCGGTTCACT
 15 GGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGCCTGAAGATGTT
 GCAACTTATTACTGTCAAAGTATAACAGTGCCCCGCTCACTTTCGGCGGAGGGACCAAG
 GTGGAGATCAAACGA 3' (SEQ ID NO:100)

Amino acid sequence

20 5' LTQSPSSLSASVRDRVITC RASQDISNYLA WYQQKPGKVPNLLIY AASTLQS GVPSRFS
 GSGSGTDFLTITISLQPEDVATYYC QKYNAPLT FGGGTKVEIKR 3' (SEQ ID NO:101)

TABLE 29. 2.15.1 Light chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FRI	LTQSPSSLSASVRDRVITC	1-20	SEQ ID NO:102
CDR1	RASQDISNYLA	21-31	SEQ ID NO:103
FR2	WYQQKPGKVPNLLIY	32-46	SEQ ID NO:104
CDR2	AASTLQ	47-52	SEQ ID NO:105
FR3	GVPSRFSGSGSGTDFLTITISLQPEDVATYYC	53-84	SEQ ID NO:106
CDR3	QKYNAPLT	85-93	SEQ ID NO:107
FR4	FGGGTKVEIKR	94-104	SEQ ID NO:108

*AA Residues of SEQ ID NO:101

25

Antibody -2.16.1**Heavy chain variable region****Nucleotide sequence**

30 5' CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTC
 TCCTGTGCAGCCTCTGGATTACCTTCAGTGACTACTACATGACCTGGATCCGCCAGGCT
 CCAGGGAAGGGGCTGGAGTGGGTTTCATACATTAGTATTAGTGGTAGTATCACACTAC
 GCAGACTCAGTGAAGGGCCGATTACCATGTCCAGGGACAACGCCAAGAACTCACTGTAT
 CTGCAAATGAACAGCCTGAGAGCCGAGGACACGCCCGTGTATTACTGTGCGAGAGACGGA
 GCAGCAGCTGGTACGGATGCTTTTGATATCTGGGGCCACGGGACAAAGGTCACCGTCTCT
 35 TCAGCC 3' (SEQ ID NO:109)

Amino acid sequence

5' QVQLVESGGGLVKPGSLRLSCAAS GFTFSDYYMT WIRQAPGKGLEWVS YISISGSITHY

ADSVKG RFTMSRDNAKNSLYLQMNSLRAEDTAVYYCAR DGAAAGTDAFDI WGHGTVTVSSA 3'
(SEQ ID NO:110)

TABLE 30. 2.16.1 Heavy chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FR1	QVQLVESGGGLVKPGGSLRLSCAAS	1-25	SEQ ID NO:111
CDR1	GFTFSDYYMT	26-35	SEQ ID NO:112
FR2	WIRQAPGKGLEWVS	36-49	SEQ ID NO:113
CDR2	YISISGSITHYADSVKG	50-66	SEQ ID NO:114
FR3	RFTMSRDNAKNSLYLQMNSLRAEDTAVYYCAR	67-98	SEQ ID NO:115
CDR3	DGAAAGTDAFDI	99-110	SEQ ID NO:116
FR4	WGHGTVTVSSA	111-122	SEQ ID NO:117

5 *AA Residues of SEQ ID NO:110

Light chain variable region

Nucleotide sequence

10 5' GAGATAGTGATGACGCAGTCTCCAGCCACCCTATCTGTGTCTCCAGGGACAGAGCCACCCTCTCCTGCAGG
GCCAGTCAGAATGTTAGCAGCAACTTGGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTT
TGGTGCATCCACCAGGGCCACTGGTATCCCAGCCAGGTTCACTGGCAGTGGGTCTGGGACAGAGTTCACTCTCA
CCATCAGCAGCCTACAGTCTGAAGATTTGTCAGTTTATTACTGTCAGCAGTATCATTACTGGCCCACTTTTCGGC
CCTGGGACCAAAGTGGATACAAACGA 3' (SEQ ID NO:118)

Amino acid sequence

15 5' EIVMTQSPATLSVSPGDRATLSC RASQNVSSNLA WYQQKPGQAPRLIF GASTRAT
GIPARFSGSGSGTEFTLTISLQSEDFAVYYC QQYHYWPT FGPGTKVDIKR 3' (SEQ ID NO:119)

TABLE 31. 2.16.1 Light chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FR1	EIVMTQSPATLSVSPGDRATLSC	1-23	SEQ ID NO:120
CDR1	RASQNVSSNLA	24-34	SEQ ID NO:121
FR2	WYQQKPGQAPRLIF	35-49	SEQ ID NO:122
CDR2	GASTRAT	50-56	SEQ ID NO:123
FR3	GIPARFSGSGSGTEFTLTISLQSEDFAVYYC	57-88	SEQ ID NO:124
CDR3	QQYHYWPT	89-96	SEQ ID NO:125
FR4	FGPGTKVDIKR	97-107	SEQ ID NO:126

*AA Residues of SEQ ID NO:119

20

Antibody -2.17.1

Heavy chain variable region

Nucleotide sequence

25 5' CAGCTGGTGCACTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCG
AAGGCTTCTGGATACACCTTCACCGGCTTCTATATGCACTGGGTGCGACAGACCCCTGGA
CAAGGGCTTGAGTGGATGGGATGGATCAACCCTAACAGTGGTGGCACATATTATGTACAG
AAGTTTCAGGGCAGGGTCACCATGACCAGGGACACGTCCATCAGCACAGTCTACATGGAG
CTGAGCAGGTTGAGATCTGACGACACGGCCGTATATTACTGTGCGAGAGATGGGTATAGC
AGTGGAGAGGACTGGTTCGACCCCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCC 3' (SEQ ID
30 NO:127)

Amino acid sequence

35 5' QLVSQGAIEVKKPGASVKVSKAS GYFTGFYMH WVRQTPGGGLEWVG WINPNSGGTYIVQ
KFQG RVTMTRDTSISTVYMELSRLSDDTAVYYCAR DGYSSGEDWDFP WQGTLTVTVSSA 3' (SEQ
ID NO:128)

TABLE 32. 2.17.1 Heavy chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FRI	QLVQSGAEVKKPGASVKVSCKAS	1-23	SEQ ID NO:129
CDR1	GYTFTGFYMH	24-33	SEQ ID NO:130
FR2	WVRQTPGQGLEWMG	34-47	SEQ ID NO:131
CDR2	WINPNSGGTYVYVQKFQG	48-64	SEQ ID NO:132
FR3	RVTMTRDTSISTVYMELSLRSDDTAVYYCAR	65-96	SEQ ID NO:133
CDR3	DGYSSGEDWFDP	97-108	SEQ ID NO:134
FR4	WGQGLTVTVSSA	109-120	SEQ ID NO:135

*AA Residues of SEQ ID NO:128

5 **Light chain variable region****Nucleotide sequence**

5' GATATTGTGATGACCCAGACTCCACTCTCTCTGTCCGTCACCCCTGGACAGCCGGCCTCCATCTCCTGCAAG
TCTAGTCAGAGCCTCCTGCATAGTGGTGGAAAGACCTATTTGTATTGGTACCTGCAGAGGCCAGGCCAGCCTCC
ACAGCTCCTGATCTATGAAGTTTCCAACCGTTTCTCTGGAGTGCCAGATAGGTTCACTGGCAGCGGGTCAGGGA
10 CAGATTTACACTGAAATCAGCCGGGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAAGTATACAC
CTTCCGCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAACGA 3' (SEQ ID NO:136)

Amino acid sequence

5' DIVMTQTPLSLSVTPGQPASISC KSSQSLHSGGKTYLY WYLQRPQPQLLIY EVSNRFS
15 GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC MQSIHLPLT FGGGTKVEIKR 3' (SEQ ID NO:137)

TABLE 33. 2.17.1 Light chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FRI	DIVMTQTPLSLSVTPGQPASISC	1-23	SEQ ID NO:138
CDR1	KSSQSLHSGGKTYLY	24-39	SEQ ID NO:139
FR2	WYLQRPQPQLLIY	40-54	SEQ ID NO:140
CDR2	EVSNRFS	55-61	SEQ ID NO:141
FR3	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	62-93	SEQ ID NO:142
CDR3	MQSIHLPLT	94-102	SEQ ID NO:143
FR4	FGGGTKVEIKR	103-113	SEQ ID NO:144

*AA Residues of SEQ ID NO:137

Antibody -2.21.120 **Heavy chain variable region****Nucleotide sequence**

5' CAGGTGCAGCTGGAGCAGTCGGGGGGAGGCTGGTCAAGCCTGGGGGGTCCCTGAGATTG
TCCTGTGCAGCCTCTGGATTACCTTCAGTAGCTATAGCATGAAGTGGGTCCGCCAGGCT
CCAGGGAAGGGGCTGGAGTGGGTCTCATTCACTAGTAGTAGTAGTTACATATACTAC
25 GCAGACTCAGTGAAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACTCACTGTAT
CTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGAGGAC
TGGGTGGGAGCTACCTTTGACTACTGGGGCCAGGGAACCTGGTCACCGTCTCCTCAGCC 3' (SEQ ID
NO:145)

Amino acid sequence

30 5' QVQLEQSGGGLVKPGGSLRFSCAAS GFTFSSYSMN WVRQAPGKGLEWVS FISSSSSYIYY
ADSVKG RFTISRDNAKNSLYLQMNLSRAEDTAVYYCAR EDWVGATFDY WGQGLTVTVSSA 3' (SEQ
ID NO:146)

TABLE 34. 2.21.1 Heavy chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FRI	QVQLEQSGGGLVKPGGSLRFSCAAS	1-25	SEQ ID NO:147
CDR1	GFTFSSYSMN	26-35	SEQ ID NO:148
FR2	WVRQAPGKGLEWVS	36-49	SEQ ID NO:149
CDR2	FISSSSYIYYADSVKG	50-66	SEQ ID NO:150
FR3	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR	67-98	SEQ ID NO:151
CDR3	EDWVGATFDY	99-108	SEQ ID NO:152
FR4	WGQGLTVTVSSA	109-120	SEQ ID NO:153

*AA Residues of SEQ ID NO:146

Light chain variable region**5 Nucleotide sequence**

5' GACATTCAGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
 ATCACTTGTTCGGCGCAGTCAGGGCATTAGGAATTATTTAGCCTGGTATCAGCAGAAACCA
 GGGAAAGTTCCTAAGCTCCTGATCTATGCTGCTTCCGCTTTGAAATTAGGGGTCCCCTCT
 CGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGCCT
 10 GAAGATGTTGCAACTTATTACTGTCAAAGTATAACAGTGCCCCGATCACCTTCGGCCAA
 GGGACACGACTGGACATTAAACGA 3' (SEQ ID NO:154)

Amino acid sequence

5' DIQLTQSPSSLSASVGDRTITC RASQGIRNYLA WYQQKPGKVPKLLIY AASALKL GVPS
 15 RFSGSGSGTDFTLTISLQPEDVATYYC QKYNAPIT FGQGRDLIKR 3' (SEQ ID NO:155)

TABLE 35. 2.21.1 Light chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FRI	DIQLTQSPSSLSASVGDRTITC	1-23	SEQ ID NO:156
CDR1	RASQGIRNYLA	24-34	SEQ ID NO:157
FR2	WYQQKPGKVPKLLIY	35-49	SEQ ID NO:158
CDR2	AASALKL	50-56	SEQ ID NO:159
FR3	GVPSRFSGSGSGTDFTLTISLQPEDVATYYC	57-88	SEQ ID NO:160
CDR3	QKYNAPIT	89-97	SEQ ID NO:161
FR4	FGQGRDLIKR	98-108	SEQ ID NO:162

*AA Residues of SEQ ID NO:155

Antibody -2.22.1**20 Heavy chain variable region****Nucleotide sequence**

5' CAGGTGCAGCTGGAGCAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGAACCTGTCCCTC
 ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGGTTATTTCTGGAGCTGGATCCGC
 CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAACACCTAC
 25 TACAACCCGTCCTCAAGAGTCGAGTTACCATATCAGTTGACACGTCTAAGAACCAGTTC
 TCCCTGAAACTGAGCTCTGTGACTGCCGCGACACGGCCGTGTATTACTGTGCGAGAGAC
 TATTACTATGATACTAGTGGTTTTCCTACCGTTACGACTGGTACTACGGTATGGACGTC
 TGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGCC 3' (SEQ ID NO:163)

Amino acid sequence

30 5' QVQLEQSGPGLVKPSQNLSTCTVS GGSISSGGYFWS WIRQHPGKGLEWIG YIYYSGNTY
 YNPSLKS RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR DYYYDTSGFSYRYDWYGMVDV
 WGQGTTVTVSSA 3' (SEQ ID NO:164)

TABLE 36. 2.22.1 Heavy chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FR1	QVQLEQSGPGLVKPSQNLSTCTVS	1-25	SEQ ID NO:165
CDR1	GGSISSGGYFWS	26-37	SEQ ID NO:166
FR2	WIRQHPGKGLEWIG	38-51	SEQ ID NO:167
CDR2	YIYYSGNTYYNPSLKS	52-67	SEQ ID NO:168
FR3	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	68-99	SEQ ID NO:169
CDR3	DYYYDTSGFSYRYDWYYGMDV	100-120	SEQ ID NO:170
FR4	WGQGTITVTVSSA	121-132	SEQ ID NO:171

*AA Residues of SEQ ID NO:164

Light chain variable region**5 Nucleotide sequence**

5' GACATCCAGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
 ATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTAGGCTGGTATCAGCAGAAACCA
 GGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTGCAAATGGGGTCCCATCA
 AGGTTCAAGCGCAGTGGATCTGGACAGAATTCCTCTCACAATCAGCAGCCTGCAGCCT
 10 GAAGATTTTGCAACTTATTACTGTCTACAACATAATACTTACCCGGCGTTCGGCCAAGGG
 ACCAAGGTGGAATCAAACGA 3' (SEQ ID NO:172)

Amino acid sequence

5' DIQLTQSPSSLSASVGDRVTITC RASQGIKNDLG WYQKPGKAPKRLIY AASSLQN GVPS
 15 RFSGSGSGTEFTLTISLQPEDFATYYC LQHNTYPA FGQGTKVEIKR 3' (SEQ ID NO:173)

TABLE 37. 2.22.1 Light chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FR1	DIQLTQSPSSLSASVGDRVTITC	1-23	SEQ ID NO:174
CDR1	RASQGIKNDLG	24-34	SEQ ID NO:175
FR2	WYQKPGKAPKRLIY	35-49	SEQ ID NO:176
CDR2	AASSLQN	50-56	SEQ ID NO:177
FR3	GVPSRFSGSGSGTEFTLTISLQPEDFATYYC	57-88	SEQ ID NO:178
CDR3	LQHNTYPA	89-97	SEQ ID NO:179
FR4	FGQGTKVEIKR	98-108	SEQ ID NO:180

*AA Residues of SEQ ID NO:173

Antibody - 2.24.1**20 Heavy chain variable region****Nucleotide sequence**

5' CAGCTGGTGCAGTCTGGAGCAGAAGTGAAGAGCCCGGGGAGTCTCTGAAGATCTCCTGT
 CAGGGTTCTGGATACATCTTTACCAACTACTGGATCGGCTGGGTGCGCCAGATGCCCGGG
 AAAGGCCTGGAGTGGATGGGGTCATCTATCCTGATGACTCTGATACCAGATACAGCCCCG
 25 TCCTTCCAAGGCCAGGTACCATCTCAGCCGACAAGTCCATCAGCACCAGCTACCTGCAG
 TGGAGCAGCCTGAAGGCCTCGGACACCGCCATATATTACTGTGCGAGACAAAATGGCTA
 CAACACCCCTTTGACTACTGGGGCCAGGAACCCCTGGTCACCGTCTCCTCAGCC 3' (SEQ ID NO:181)

Amino acid sequence

5' QLVQSGAEVKKPGESLKISQGS GYIFTNYWIG WVRQMPGKGLEWMG VIYPDDSDTRYSP
 30 SFQG QVTISADKSISTAYLQWSSLKASDTAIYYCAR QKWLQHPFDY WGQGTITVTVSSA 3' (SEQ ID
 NO:182)

TABLE 38. 2.24.1 Heavy chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FR1	QLVQSGAEVKKPGESLKISCQGS	1-23	SEQ ID NO:183
CDR1	GYIFTNYWIG	24-33	SEQ ID NO:184
FR2	WVRQMPGKGLEWMG	34-47	SEQ ID NO:185
CDR2	VJYPDDSDTRYSPSFQG	48-64	SEQ ID NO:186
FR3	QVTISADKSISTAYLQWSSLKASDTAIYYCAR	65-96	SEQ ID NO:187
CDR3	QKWLQHPFDY	97-106	SEQ ID NO:188
FR4	WGQGLTVTVSSA	107-118	SEQ ID NO:189

*AA Residues of SEQ ID NO:182

Light chain variable region5 **Nucleotide sequence**

5' GAAATTGTGTTGACGCAGTCACCAGGCACCCTGTCTTGTCTCCAGGGGAAAGAGTCACC
 CTCTCATGCAGGGCCAGTCAGAGTGTTAGCAGCAGATACTTAGCCTGGTACCAGCAGAAA
 CCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCA
 GACAGGTTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAG
 10 CCTGAAGATTTTGCAGTTTATTACTGTGACAGTATGGTAGCTCACCTCGGACGTTTCGGC
 CAAGGGACCAAGGTGGAAATCAAACGA 3' (SEQ ID NO:190)

Amino acid sequence

5' EIVLTQSPGTLSPGERVTLSC RASQSVSSRYLA WYQQKPGQAPRLLIY GASSRAT GIP
 15 DRFSGSGSGTDFTLTISRLEPEDFAVYYC QYGSSPRT FGQGTKVEIKR 3' (SEQ ID NO:191)

TABLE 39. 2.24.1 Light chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FR1	EIVLTQSPGTLSPGERVTLSC	1-23	SEQ ID NO:192
CDR1	RASQSVSSRYLA	24-35	SEQ ID NO:193
FR2	WYQQKPGQAPRLLIY	36-50	SEQ ID NO:194
CDR2	GASSRAT	51-57	SEQ ID NO:195
FR3	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYY	58-88	SEQ ID NO:196
CDR3	QYGSSPRT	89-97	SEQ ID NO:197
FR4	FGQGTKVEIKR	98-109	SEQ ID NO:198

*AA Residues of SEQ ID NO:191

Antibody -2.3.120 **Heavy chain variable region****Nucleotide sequence**

5'CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCC
 TGCAAGGCTTCTGGATACACCTTCAACCGCTACTATATGCACTGGGTGCGACAGGCCCTGGACA
 AGGGCTTGAGTGGATGGGATGGATCAACCCTAACAGTGGTGGCACAACCTATGCACAGAAGTTT
 25 CAGGACAGGGTCACCATGACCAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAGCAGGC
 TGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGATTCTTTGTTTCGGGGAGTCTCCTC
 TACTTTGACTACTGGGGCCAGGGAACCTGGTCACCGTCTCCTCAGCC 3' (SEQ ID NO:199)

Amino acid sequence

5' QVQLVQSGAEVKKPGASVKVSKASGYTFTGYMHVWRQAPGQGLEWMGWINPNSGGTNYAQKFQD
 30 RVTMTRDTSISTAYMELSRRLSDDAVYYCARDFFGSGSLLYFDYWGQGLTVTVSSA 3' (SEQ ID
 NO:200)

TABLE 40. 2.3.1 Heavy chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FR1	QVQLVQSGAEVKKPGASVKVSKAS	1-25	SEQ ID NO:201
CDR1	GYTFTGYMH	26-35	SEQ ID NO:202
FR2	WVRQAPGQGLEWMG	36-49	SEQ ID NO:203
CDR2	WINPNSGGTNYAQKFQD	50-66	SEQ ID NO:204
FR3	RVTMTRDTSISTAYMELSRSDDTAVYYCAR	67-98	SEQ ID NO:205
CDR3	DFFGSGSLLYFDY	99-111	SEQ ID NO:206
FR4	WGQGLVTVSSA	112-123	SEQ ID NO:207

*AA Residues of SEQ ID NO:200

Light chain variable region5 **Nucleotide sequence**

5' GATATTGTGATGACCCAGACTCCACTCTCTGTGCCGTACCCCTGGACAGCCGGCCTCCATCTCCTGCAAG
TCTAGTCAGAGCCTCCTGCATAGTGGTGGAAAGACCTATTTGTATTGGTACCTGCAGAGGCCAGGCCAGCCTCC
ACAGCTCCTGATCTATGAAGTTCCAACCGGTTCTCTGGAGTGCCAGATAGGTTCACTGGCAGCGGGTCAGGGA
CAGATTTCACTGAAAATCAGCCGGGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAAGTATACAC
CTTCCGCTCACTTTCCGGCGAGGGACCAAGGTGGAGATCAAACGA 3' (SEQ ID NO:208)

10 **Amino acid sequence**

5' DIVMTQTPLSLSVTPGQPASISC KSSQSLHSGGKTYLY WYLQRPQPQLLIY EVSNRFS
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC MQSIHLPLT FGGGTKVEIKR 3' (SEQ ID NO:209)

15 **TABLE 41. 2.3.1 Light chain V region domains.**

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FR1	DIVMTQTPLSLSVTPGQPASISC	1-23	SEQ ID NO:210
CDR1	KSSQSLHSGGKTYLY	24-39	SEQ ID NO:211
FR2	WYLQRPQPQLLIY	40-54	SEQ ID NO:212
CDR2	EVSNRFS	55-61	SEQ ID NO:213
FR3	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	62-93	SEQ ID NO:214
CDR3	MQSIHLPLT	94-102	SEQ ID NO:215
FR4	FGGGTKVEIKR	103-113	SEQ ID NO:216

*AA Residues of SEQ ID NO:209

Antibody -2.6.1**Heavy chain variable region**20 **Nucleotide sequence**

5' CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCC
TGCAAGGCTTCTGGATACACCTTCACCGGCTACTATATGCACTGGGTGCGACAGGCCCTGGACA
AGGGCTTGAGTGGATGGATGGATCAACCCTAACAGTGGTGGCACAACCTATGCACAGAAAGTTT
CAGGACAGGGTCACCATGACCAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAGCAGGC
25 TGAGATCTGACGACACGGCCGTGATTACTGTGCGAGAGATTTCTTTGGTTCCGGGGAGTCTCCTC
TACTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCC 3' (SEQ ID NO:309)

Amino acid sequence

5' QVQLVQSGAEVKKPGASVKVSKAS GYTFTGYMH WVRQAPGQGLEWMG WINPNSGGTNYAQKFQD
RVTMTRDTSISTAYMELSRSDDTAVYYCAR DFFGSGSLLYFDY WGQGLVTVSSA 3' (SEQ ID
30 NO:310)

TABLE 42. 2.6.1 Heavy chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FR1	QVQLVQSGAEVKKPGASVKVSKAS	1-25	311
CDR1	GYTFTGYMH	26-35	312
FR2	WVRQAPGQGLEWMG	36-49	313
CDR2	WINPNSGGTNYAQKFQD	50-66	314
FR3	RVTMTRDTSISTAYMELSRLRSDDTAVYYCAR	67-98	315
CDR3	DFFGSGSLLYFDY	99-112	316
FR4	WGQGLTVTVSSA	113-124	317

*AA Residues of SEQ ID NO:310

Light chain variable region5 **Nucleotide sequence**

5' GATATTGTGATGACCCAGACTCCACTCTCTCTGTCCGTACCCCTGGACAGCCGGCCTCCATCTCCTGCAAG
TCTAGTCAGAGCCTCCTGCATAGTGGTGGAAAGACCTATTTGTATTGGTACCTGCAGAGGCCAGGCCAGCCTCC
ACAGCTCCTGATCTATGAAGTTTCCAACCGGTTCTCTGGAGTGCCAGATAGGTTTCAGTGGCAGCGGGTCAGGGA
CAGATTTCACTGAAAATCAGCCGGGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAAGTATACAC
CTTCCGCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAACGA 3' (SEQ ID NO:318)

10 **Amino acid sequence**

5' DIVMTQTPLSLSVTPGQPASISC KSSQSLHSGGKTYLY WYLQRPQPPQLLIY EVSNRFS
GVPDFRSGSGSGTDFTLKISRVEAEDVGVYYC MQSIHLPLT FGGGTKVEIKR 3' (SEQ ID NO:319)

15 TABLE 43. 2.6.1 Light chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FR1	DIVMTQTPLSLSVTPGQPASISC	1-23	320
CDR1	KSSQSLHSGGKTYLY	24-39	321
FR2	WYLQRPQPPQLLIY	40-54	322
CDR2	EVSNRFS	55-61	323
FR3	GVPDFRSGSGSGTDFTLKISRVEAEDVGVYYC	62-93	324
CDR3	MQSIHLPLT	94-102	325
FR4	FGGGTKVEIKR	103-113	326

*AA Residues of SEQ ID NO:319

Antibody -2.7.1**Heavy chain variable region**20 **Nucleotide sequence**

5' CAGGTGCAGCTGGAGCAGTCGGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTC
TCCTGTGCAGCGTCTGGATTACCTTCAATAACTATGGCATGCACTGGGTCCGCCAGGCT
CCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTATGATGGAAGTAATAAATACTAT
GCAGACTCCGTGAAGGGCCGATTACCATCTCCAGAGACAATTCCAAGAACACGCTGTAT
25 CTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAAAGATGAG
GAATACTACTATGTTTCGGGGCTTGACTACTGGGGCCAGGAACCCTGGTCACCGTCTCC
TCAGCC 3' (SEQ ID NO:217)

Amino acid sequence

5' QVQLEQSGGGVVQGRSLRLSCAAS GFTFNNGMH WVRQAPGKGLEWVA VIWYDGSNKYY
30 ADSVKG RFTISRDNKNTLYLQMNLSRAEDTAVYYCAK DEEYIYVSGLDY WGQGLTVTVSSA 3'
(SEQ ID NO:218)

TABLE 44. 2.7.1 Heavy chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FRI	QVQLEQSGGGVVQGRSLRLSCAAS	1-25	SEQ ID NO:219
CDR1	GFTFNNGMH	26-35	SEQ ID NO:220
FR2	WVRQAPGKGLEWVA	36-49	SEQ ID NO:221
CDR2	VIWYDGSNKYYADSVKG	50-66	SEQ ID NO:222
FR3	RFTISRDNKNTLYLQMNSLRAEDTAVYYCAK	67-98	SEQ ID NO:223
CDR3	DEEYVVVSGLDY	99-110	SEQ ID NO:224
FR4	WGQGTLLVTVSSA	111-122	SEQ ID NO:225

*AA Residues of SEQ ID NO:218

5 Light chain variable region

Nucleotide sequence

5' CTGACTCAGTCTCCATCTCCCTGTCTGCATCTGTAAGAGACAGAGTCACCATCACTTGC
 CGGGCGAGTCAGGACATTAGCAATTATTTAGCCTGGTATCAGCAGAAACCAGGAAAGTT
 CCTAATCTCCTGATCTATGCTGCATCCACTTTGCAATCAGGGGTCCCATCTCGGTTTCAGT
 10 GGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGCCTGAAGATGTT
 GCAACTTATTACTGTCAAAGTATAACAGTGCCCGCTCACTTTTCGGCGGAGGGACCAAG
 GTGGAGATCAAACGA 3' (SEQ ID NO:226)

Amino acid sequence

5' LTQSPSSLSASVRDRVTITC RASQDISNYLA WYQQKPGKVPNLLIY AASTLQS GVPSRFS
 15 GSGSGTDFLTITSLQPEDVATYYC QKYNAPLT FGGGTKVEIKR 3' (SEQ ID NO:227)

TABLE 45. 2.7.1 Light chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FRI	LTQSPSSLSASVRDRVTITC	1-20	SEQ ID NO:228
CDR1	RASQDISNYLA	21-31	SEQ ID NO:229
FR2	WYQQKPGKVPNLLIY	32-46	SEQ ID NO:230
CDR2	AASTLQ	47-52	SEQ ID NO:231
FR3	GVPSRFSGSGTDFLTITSLQPEDVATYYC	53-84	SEQ ID NO:232
CDR3	QKYNAPLT	85-93	SEQ ID NO:233
FR4	FGGGTKVEIKR	94-104	SEQ ID NO:234

*AA Residues of SEQ ID NO:227

20 Antibody - 2.8.1

Heavy chain variable region

Nucleotide sequence

5' CAGATCACCTTGAAGGAGTCTGGTCTACGCTGGTGACACCCACACAGACCCTCACGCTG
 ACCTGCACCTTCTCTGGGTTCTCACTCAGCACTGGTGGAATGGGTGTGGGCTGGATCCGT
 25 CAGCCCCCAGGAAAGGCCCTGGACTGGCTTACACTCATTTATTGGAATGATGATAAGCAC
 TACAGCCCATCTCTGAAGAGCAGGCTTACCATACCAAGGACACCTCCAAAAACCAGGTG
 GTCCTTAGAATGACCAACATGGACCCCTGTGGACACAGCCACTTATTACTGTGCACACCTG
 CATTACGATATTTGACTGGTTTAACTTTGACTACTGGGGCCAGGGAACCCTGGTCACC
 GTCTCCTCAGCC 3' (SEQ ID NO:235)

30 Amino acid sequence

5' QITLKESGPTLVPTQTLLTCTFS GFSLSSTGGMVG WIRQPPGKALDWLT LIYWDDKH
 YSPSLKS RLITTKDTSKNQVLRMTNMDPVDATATYYCAH LHYDILTGFNFY WGQGTLLVTVSSA 3'
 (SEQ ID NO:236)

TABLE 46. 2.8.1 Heavy chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FR1	QITLKESGPTLVPTQTLTLTCTFS	1-25	SEQ ID NO:237
CDR1	GFSLSTGGMGVG	26-37	SEQ ID NO:238
FR2	WIRQPPGKALDWLT	38-51	SEQ ID NO:239
CDR2	LIYWNDKHYSPSLKS	52-67	SEQ ID NO:240
FR3	RLTITKDTSKNQVVLMTNMDPVDATYYCAH	68-99	SEQ ID NO:241
CDR3	LHYDILTGFNFDY	100-112	SEQ ID NO:242
FR4	WGQGLTVTVSSA	113-124	SEQ ID NO:243

*AA Residues of SEQ ID NO:236

5 **Light chain variable region****Nucleotide sequence**

5' GATATTGTGATGACCCAGACTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCCATCTCCTGCAGG
TCTAGTCAGAGCCTCTTGGATAGTGATGATGGAACACCTATTTGGACTGGTACCTGCAGAAGCCAGGGCAGTC
TCCACAGCTCCTGATCTATACGCTTTCCTATCGGGCCTCTGGAGTCCCAGACAGGTTTCAGTGGCAGTGGGTGAG
10 GCACTGATTTCACTGAAAATCAGCAGGCTGGAGGCTGAGGATGTTGGAGTTTATTACTGCATGCAACGTATA
GAGTTCCGCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAACGA 3' (SEQ ID NO:244)

Amino acid sequence

5' DIVMTQTPLSLPVTGPGEASISC RSSQSLLDSDDGNTYLD WYLQKPGQSPQLLIY TLSYRAS
15 GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC MQRIEFPLT FGGGTKVEIKR 3' (SEQ ID NO:245)

TABLE 47. 2.8.1 Light chain V region domains.

REGION	SEQUENCE	AA RESIDUES*	SEQ ID
FR1	DIVMTQTPLSLPVTGPGEASISC	1-23	SEQ ID NO:246
CDR1	RSSQSLLDSDDGNTYLD	24-40	SEQ ID NO:247
FR2	WYLQKPGQSPQLLIY	41-55	SEQ ID NO:248
CDR2	TLSYRAS	56-62	SEQ ID NO:249
FR3	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	63-94	SEQ ID NO:250
CDR3	MQRIEFPLT	95-103	SEQ ID NO:251
FR4	FGGGTKVEIKR	103-114	SEQ ID NO:252

*AA Residues of SEQ ID NO:245

Example 16: Use of anti-GPNMB Antibodies as a Diagnostic Agent20 **Detection of GPNMB antigen in a sample:**

The following is a protocol for an Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of GPNMB antigen in a sample. In the assay, wells of a microtiter plate, such as a 96-well microtiter plate or a 384-well microtiter plate, are adsorbed for several hours with a first fully human monoclonal antibody directed against GPNMB. The
25 immobilized antibody serves as a capture antibody for any of the GPNMB that may be present in a test sample. The wells are rinsed and treated with a blocking agent such as milk protein or albumin to prevent nonspecific adsorption of the analyte.

Subsequently the wells are treated with a test sample suspected of containing the GPNMB antigen, or with a solution containing a standard amount of GPNMB antigen.

Such a sample may be, for example, a serum sample from a subject suspected of having levels of circulating GPNMB considered to be diagnostic of a pathology.

After rinsing away the test sample or standard, the wells are treated with a second fully human monoclonal anti-GPNMB antibody that is labeled by conjugation with biotin.

- 5 The labeled anti-GPNMB antibody serves as a detecting antibody. After rinsing away excess second antibody, the wells are treated with avidin-conjugated horseradish peroxidase (HRP) and a suitable chromogenic substrate. The concentration of the antigen in the test samples is determined by comparison with a standard curve developed from the standard samples.

- 10 This ELISA assay provides a highly specific and very sensitive assay for the detection of the GPNMB antigen in a test sample.

Determination of GPNMB antigen concentration in patients:

- A sandwich ELISA can also be used to quantify GPNMB levels in human serum. The 2 fully human monoclonal anti-GPNMB antibodies used in the sandwich ELISA, recognize different epitopes on the GPNMB molecule. The ELISA is performed as follows:
- 15 50 μ l of capture anti-GPNMB antibody in coating buffer (0.1 M NaHCO₃, pH 9.6) at a concentration of 2 μ g/mL is coated on ELISA plates (Fisher). After incubation at 4°C overnight, the plates are treated with 200 μ l of blocking buffer (0.5% BSA, 0.1% Tween 20, 0.01% Thimerosal in PBS) for 1 hr at 25°C. The plates are washed (3x) using 0.05% Tween 20 in PBS (washing buffer, WB). Normal or patient sera (Clinomics, Bioreclamation) are diluted in blocking buffer containing 50% human serum. The plates are incubated with serum samples overnight at 4°C, washed with WB, and then incubated with 100 μ l/well of biotinylated detection anti-GPNMB antibody for 1 hr at 25°C. After washing, the plates are incubated with HRP-Streptavidin for 15 min, washed as before, and
- 20 then treated with 100 μ l/well of o-phenylenediamine in H₂O₂ (Sigma developing solution) for color generation. The reaction is stopped with 50 μ l/well of H₂SO₄ (2M) and analyzed using an ELISA plate reader at 492 nm. Concentration of GPNMB antigen in serum samples is calculated by comparison to dilutions of purified GPNMB antigen using a four parameter curve fitting program.

- 25 Staging of cancer in a patient:

It will be appreciated that based on the results set forth and discussed in the above diagnostic examples, it is possible to stage a cancer in a subject based on expression levels

of the GPNMB antigen. For a given type of cancer (e.g., melanoma), samples of blood are taken from subjects diagnosed as being at various stages in the progression of the disease, and/or at various points in the therapeutic treatment of the cancer. The concentration of the GPNMB antigen present in the blood samples is determined using a method that specifically
5 determines the amount of the antigen that is present. Such a method includes an ELISA method, such as the method described in the previous diagnostic examples. Using a population of samples that provides statistically significant results for each stage of progression or therapy, a range of concentrations of the antigen that may be considered characteristic of each stage is designated.

10 In order to stage the progression of the cancer in a subject under study, or to characterize the response of the subject to a course of therapy, a sample of blood is taken from the subject and the concentration of the GPNMB antigen present in the sample is determined. The concentration so obtained is used to identify in which range of concentrations the value falls. The range so identified correlates with a stage of progression
15 or a stage of therapy identified in the various populations of diagnosed subjects, thereby providing a stage in the subject under study.

Example 17: Diagnosing Cancer With Antibodies Against GPNMB

A subject suspected of having an ovarian cancer tumor is identified and a tissue sample from the suspected tumor is removed for testing. The removed tissue is then
20 contacted with anti-GPNMB antibodies having a colorimetric label. A determination is made of whether the anti-GPNMB antibodies bind specifically to the removed tissue. Binding is indicative of cancerous tissue while the absence of binding is indicative of non-cancerous tissue. The patient's condition is diagnosed accordingly to facilitate subsequent testing, counseling, and/or treatment.

25 **Example 18: Treating Cancer With Antibodies Against GPNMB**

Targeting GPNMB on tumor cells is useful to treat a subject at risk for or afflicted with cancer. Such a subject would benefit from treatment with an anti-GPNMB antibody of the present invention. Typically, antibodies are administered in an outpatient setting by weekly administration at about 0.1-1.0 mg/kg dose by slow intravenous (IV) infusion. The
30 appropriate therapeutically effective dose of an antibody is selected by a treating clinician and would range approximately from 1 μ g/kg to 20mg /kg, from 1 μ g/kg to 10 mg/kg, from 1 μ g/kg to 1mg/kg, from 10 μ g/kg to 1 mg/kg, from 10 μ g/kg to 100 μ g/kg, from 100 μ g/kg to 1 mg/kg, and from 500 μ g/kg to 5 mg/kg.

The antibodies are also used to prevent and/or to reduce severity and/or symptoms of disease associated with GPNMB-related disorders.

To test the clinical efficacy of antibodies in humans, individuals with cancer, particularly, but not limited to ovarian, lung or colon carcinoma are identified and randomized into treatment groups. Treatment groups include a group not receiving antibody treatment and groups treated with different doses of anti-GPNMB antibody. Individuals are followed prospectively and individuals receiving antibody treatment exhibit an improvement in their condition.

Example 19: The Specificity of the Anti-tumor Effects of CR011-vcMMAE (CR011-ONC-1)

The study was performed to determine the anti-tumor effects of the constituent components of the antibody-drug conjugate and its formulation and to relate these effects to the anti-tumor effects of the intact immunoconjugate.

Results:

Mice were implanted by trocar with fragments of SK-ME-2 melanoma and, after the tumors became established, treatment with CR011-vcMMAE and various components was tested to demonstrate the specificity of anti-tumor effects of this agent. Control groups, dosed with either the phosphate-buffered saline (vehicle) or the excipients of the immunoconjugate preparation (3% DMSO, sucrose, phosphate medium) steadily increased in tumor size to a maximum of 2,000 mg, at which time they were removed from the study. No apparent or statistically significant anti-tumor effects were observed. However, CR011-vcMMAE treatment (at 5 mg/kg/treatment, q4d x4) produced measurable inhibition after the first 2 doses. Tumor growth inhibition continued until no discernible tumor was detected in all 6 of the test animals (Figure 4). In preliminary studies, tumor regression was complete and was not followed by regrowth of the tumor despite lengthy observation periods (up to 200 days).

Conclusions:

The regressions produced by the immunoconjugate were not due to the individual components of the immunoconjugate nor to components of the formulation of that immunoconjugate. This is demonstrated by the lack of tumor growth inhibition after treatment with CR011 antibody alone (group 3) or free monomethylauristatin E (group 4), where the doses applied were identical to that contained in the intact immunoconjugate. Furthermore, the lack of anti-tumor effects noted with free MMAE suggests that anti-tumor

effects from MMAE as a result of slow release from the antibody-drug conjugate may not explain the anti-tumor effects of the immunoconjugate. Release of MMAE from antibody-MMAE conjugates has been shown to be a very slow process *in vivo* ($T_{1/2} = 6.0$ days in the case of the anti-CD30 antibody-Auristatin E immunoconjugate (Sanderson *et al.*, Clin. Cancer Res. 11: 843-852 (2005)) and would provide for plasma or serum concentrations that would be considerably lower than the "bolus" doses used in this study, which were ineffective at slowing the growth of the human melanoma xenografts.

Example 20: CR011-vcMMAE Inhibits the Growth of Human SK-MEL-5 Melanoma Xenografts Leading to Complete Regression of Established Melanoma Tumors in Athymic Mice (CR011-ONC-3)

This study was performed to assess the potency and therapeutic efficacy of the antibody-drug conjugate, CR011-vcMMAE, against a second model of established human melanoma, the SK-MEL-5 xenograft.

Results:

Though unrelated in origin, the SK-MEL-5 expresses GPNMB on the surface of the cell membrane and is killed by CR011-vcMMAE *in vitro*. In this study, the anti-tumor effects of the CR011 immunoconjugate were examined, along with the vehicles PBS and saline, and the reference agents vinblastine and paclitaxel. In a manner similar to the SK-MEL-2 tumor, vinblastine produced a noticeable, but not significant tumor growth inhibition ($P \leq 0.21$) when compared to saline and PBS control groups (Figure 5). Soon after the commencement of treatment with paclitaxel, however, significant tumor growth inhibition was observed ($P \leq 0.039$) at day 3 after treatment began, and this anti-tumor effect continued, producing 100 % growth inhibition (stasis). The responses of SK-MEL-5-bearing test animals to vinblastine and paclitaxel were short-lived. After cessation of treatment at the maximally tolerated doses, tumors resumed rapid, progressive growth. One long-term, tumor-free survivor occurred in the paclitaxel group and one spontaneous regression occurred in the group treated with saline.

Substantial tumor growth inhibition, as well as tumor growth delay and complete regressions occurred in SK-MEL-5 tumor-bearing animals after treatment with CR011-vcMMAE, and these effects were dose-related. At 10 mg/kg/treatment, significant anti-tumor effects were noted as early as 7 days (the equivalent of 2 treatments) after treatments began, when compared to saline ($P \leq 0.0096$), and as early as 10 days after treatment began when compared to PBS-treated controls ($P = 0.039$). In a dose-related manner, CR011-vcMMAE produced tumor growth delay leading to complete regressions of established SK-

MEL-5 melanoma xenografts (see tabular insert to Figure 5 for proportions of animals with complete regressions). Complete regressions occurred at CR011-vcMMAE doses of 2.5 mg/kg/treatment, but not at 1.25 mg/kg/treatment.

As in previous studies, no indication of toxicity by the immunconjugate occurred in treated animals as evidenced by mortality of effects on body weight or weight gain.

Conclusions:

CR011-vcMMAE exerts substantial, dose-dependent anti-tumor effects against established xenografts of the SK-MEL-5 human melanoma. After just one or two treatments significant tumor growth inhibition is noted and which leads to long-term tumor-free survivors. Complete regressions occurred at doses of ≥ 2.5 mg/kg i.v., q4d X4.

Example 21: Pharmacokinetics of CR011-vcMMAE (CR011-PK-1A)

The purpose of this study was to determine the stability of CR011-vcMMAE *in vivo* after intravenous injection, the anticipated route of clinical administration.

Materials & Methods.

The CR011 antibody component of CR011-vcMMAE was measured by a sandwich style enzyme-linked immunosorbent assay (ELISA) where serum was added to the wells of microtiter plates coated with the cognate antigen (GPNMB, CG56972-03) for the CR011 antibody, and the amount of human antibody were detected with an anti-globulin conjugated to the signal generator (horseradish peroxidase).

Results:

Pharmacokinetics. The persistence of compound availability for antibody component of CR011-vcMMAE was examined in a pharmacokinetic study in athymic mice (study CR011-PK-1, Figure 6). The serum concentration-time profile for the antibody-drug conjugate was determined in athymic mice after intravenous administration of CR011-vcMMAE and the results are presented in Figure 6. Athymic mice receiving 1 or 10 mg/kg intravenously showed dose-proportional serum concentrations over the entire span of sampling times (42 days). The concentration-time pattern was bi-phasic. The initial phase (α), however, was minor as it contributed $<2\%$ of the total AUC. Nevertheless, the compound disappeared very slowly from the peripheral blood ($T_{1/2\beta} = 10.3$ days) with serum concentrations of 1 $\mu\text{g/mL}$ and 10 $\mu\text{g/mL}$ remaining in the blood for 6 weeks after dosing.

Estimates for the pharmacokinetic parameters for CR011-vcMMAE are presented in Table 48. One parameter is noteworthy. The volume of distribution at steady state (V_{ss}) is very low, approaching the theoretical minimum; this suggests that the compound does not

distribute outside the extravascular space. The distribution pattern, as well as the β -elimination phase for CR011-vcMMAE are in good agreement with values obtained for antibodies in general (see Reviews by Mahmood and Green, Clin. Pharmacokinet 44: 331-347 (2005); or Lobo *et al.* J. Pharm. Sci. 93: 2645-2668 (2004)) and agree with values
 5 obtained for an antibody-Auristatin E immunoconjugate with comparable drug loading (Hamblett *et al.*, Clin. Cancer Res. 10: 7063-7070 (2004)).

Table 48. PK Parameters for CR011-vcMMAE after Intravenous Administration.

Parameter	Units	1 mg/kg	10 mg/kg
A	$\mu\text{g/mL}$	8.97	74.6
B	$\mu\text{g/mL}$	9.82	113
Alpha	1/h	0.179	0.0812
Beta	1/h	0.00269	0.00281
AUC	$\text{h} \cdot \mu\text{g/mL}$	3712	41210
Alpha-Half Life	h	3.88	8.531
Beta- Half Life	h	258	247
Volume	mL/kg	53.2	53.2
C _{max}	$\mu\text{g/mL}$	18.8	188
Cl	mL/h/kg	0.269	0.243
MRT	h	368	348
V _{ss}	mL/kg	99.0	84.5

Abbreviations: A: Pre-exponential constant for alpha phase; Alpha: Exponential
 10 rate constant for alpha phase; AUC Total area under the curve from 0 to infinity; B: Pre-exponential constant for beta phase; Beta: Exponential rate constant for beta phase; Cl: Total or systemic clearance; C_{max}: Maximum observed concentration; MRT: Mean residence time; Volume: Volume of central compartment; V_{ss}: Steady-state volume of distribution.

15 Estimates for pharmacokinetic parameters are presented in Table 48. One parameter is noteworthy. The volume of distribution steady state (V_{ss}) is, approaching the theoretical minimum. These data suggest that the compound did not distribute outside the extravascular space. Taken together, these data are in good agreement with data on other immunoconjugates bearing the -vcMMAE cytotoxic moiety (see Hamblett *et al.*, Clin.
 20 Cancer Res. 10: 7063-7070 (2004)).

Conclusions:

The CR011-vcMMAE antibody-drug conjugate has a serum-concentration profile which favors continuous exposure sufficient for disruption and eradication of melanoma xenografts. The immunoconjugate after i.v. administration has a sufficiently long half-life to ensure exposure of tumor cells for extended periods ($T_{1/2\beta} = 10.3$ days), and may not require frequent dosing. The durability of CR011-vcMMAE *in vivo* (e.g., athymic mice) is comparable to other Auristatin E immunoconjugates.

Example 22: The Schedule Dependency of the Anti-Tumor Effects of CR011-vcMMAE (CR011-ONC-1)

The purpose of this study was to determine the extent to which the curative anti-tumor effects of the CR011 antibody-drug conjugate are dependent on the dosing regimen and, if possible, to determine the optimum dosing interval for this xenograft model.

Materials and Methods:

The protocol for this study is presented in Table 49. To test the hypothesis that curative anti-tumor effects are influenced by the dosing schedule, the anti-tumor effects of CR011-vcMMAE were measured at 5 different dosing intervals (i.e., 0, 1, 4, 8, and 16 days between treatments) and for each dosing interval 3 dosage levels were employed (i.e., cumulative doses of 2, 8, and 32 mg/kg); for each group, $n = 6$ athymic mice.

Nota bene: Please note that, although all 5 sets of groups in this experiment (e.g., groups 5, 6, and 7 represent one set and received 32, 8, and 2 mg/kg cumulative dose, respectively) received the same cumulative doses, the first set receiving the "bolus dose" is different from the other 4 sets. The C_{max} for each group in the "bolus" set was likely four-fold higher than the C_{max} for the other 4 sets (see section on pharmacokinetics for dose-linearity after i.v. administration), since 4 sets of groups received 4 treatments, whereas the first set received only one "bolus" treatment (see column 7, Table 49 below).

TABLE 49. Protocol for the Dosing Interval Study (CR011-PHM-2).

Group	Treatment	ROA	Dose (mg/kg)	Regimen	Dosing Interval (days)	No. Treatments (n)	Cum. Dose (mg/kg)
1	Phosphate Buffered Saline			Bolus	0	1	N.A.
2	CR011-AE	i.v.	32	Bolus	0	1	32
3	CR011-AE	i.v.	8	Bolus	0	1	8
4	CR011-AE	i.v.	2	Bolus	0	1	2
5	CR011-AE	i.v.	8	qd x4	1	4	32
6	CR011-AE	i.v.	2	qd x4	1	4	8
7	CR011-AE	i.v.	0.5	qd x4	1	4	2
8	CR011-AE	i.v.	8	q4d x4	4	4	32
9	CR011-AE	i.v.	2	q4d x4	4	4	8
10	CR011-AE	i.v.	0.5	q4d x4	4	4	2
11	CR011-AE	i.v.	8	q8d x4	8	4	32
12	CR011-AE	i.v.	2	q8d x4	8	4	8
13	CR011-AE	i.v.	0.5	q8d x4	8	4	2
14	CR011-AE	i.v.	8	q16d x4	16	4	32
15	CR011-AE	i.v.	2	q16d x4	16	4	8
16	CR011-AE	i.v.	0.5	q16d x4	16	4	2
17	Excipients	i.v.	N.A.	q16d x4	16	4	N.A.

Results:

For this study, the frequency of complete regressions with long-term tumor-free survivors was determined after 5 different dosing intervals were examined empirically (*i.e.*, 0, 1, 4, 8, and 16 days between treatments). The aggregate responses for each set of groups, where a set is defined as 3 groups of graduated dosage levels but one dosage interval (groups 5, 6, and 7 represent 1 set, all of which were treated with a dosing interval of 1 day) are shown in Figure 7. The aggregate responses for test animals responding to CR011-vcMMAE appear to suggest that bolus dosing and intervals of 1 day and 4 days provide a very slight advantage to the proportion of cures, compared to longer intervals, such as 8 days and 16 days between doses. However, this effect was not significant ($P < 0.2904$). The data therefore suggest that the anti-tumor effects of CR011-vcMMAE in the SK-MEL-2 model are not schedule-dependent. This conclusion is strengthened by the fact that test animals in the bolus set (groups 2, 3, and 4), which were exposed to plasma concentrations approximately four-fold higher than any of the other groups, did not show any greater percentages of cured subjects.

The original design of this study was expanded to include an examination of the effects of various dosage levels. For each set, one group of animals received a cumulative

dose of 8 mg/kg, which, from previous studies employing a dosing interval of 4 days, provided consistent therapeutic effects leading to long-term tumor-free animals. In addition, cumulative doses of 2 mg/kg and 32 mg/kg were employed.

The effects of dosage levels, in conjunction with various dosing intervals, are presented in Figure 8. Athymic mice receiving a cumulative dose of 32 mg/kg showed complete regressions in 100 % of each group, regardless of dosing interval; that is, a cumulative dose of 32 mg/kg is schedule-independent and represents a dose which is well above that sufficient for complete regressions in 100 % of the test animals (5 groups of 6 animals/group = 30 test animals). Animals receiving 8 mg/kg cumulative dose did not demonstrate schedule dependency and showed nearly the same proportions of complete regressions (*i.e.*, 28/30 = 93%); Test animals receiving 2 mg/kg (cumulative dose), which was recognized in preliminary studies to be below the threshold for cures (using a standardized regimen of q4d X4) appeared to be schedule dependent, though this was not significant, and produced a much lower proportion of complete regressors (*i.e.*, 13%).

15 Conclusions:

The data from the dosing interval study suggests that the responses of SK-MEL-2 melanoma xenografts are not dependent on the schedule of administration of CR011-vcMMAE. While no advantage could be shown for bolus dosing or regimens with low dosing intervals, there is the suggestion that, below a certain threshold cumulative dose, there may be some advantage to combining multiple treatments into a single bolus dose.

20 Example 23: GPNMB Transcript Expression in Human Melanoma

GPNMB was recently shown to be expressed in glioblastoma and to mediate the *in vitro* and *in vivo* invasiveness of glioblastoma-derived tumor cells (see, *e.g.*, Loging *et al.*, Genome Res. 10:1393-1402 (2000); and Rich *et al.*, J. Biol. Chem. 278:15951-15975 (2003)). To confirm and extend these findings to additional cancer types, we examined the expression of GPNMB transcripts in human cancer cell lines and tissues.

Material and Methods:

Total RNA was isolated using the RNeasy kit with a DNase digestion step (Qiagen Inc., Valencia CA). RT-PCR was performed using the OneStep RT-PCR kit (Qiagen) as follows. RT : 50 °C for 45 min and 95 °C for 15 min for 1 cycle. PCR: 1 min at 95 °C, 1 min at 50 °C and 2 min at 72 °C for 30 cycles with final extension for 10 min at 72 °C. Products were separated on a 2% agarose/0.33% low melting point agarose gel and

visualized by ethidium bromide staining. The integrity of each RNA sample was verified via RT-PCR with primers designed to amplify GAPDH. Specific primers (5'-3') used were:

GPNMB: Forward-GAATTCAGAGTTAAACCTTGAG (SEQ ID NO: 327)

Reverse-CAGGAATCTGATCTGTTACCAC (SEQ ID NO: 328)

5 MART-1: Forward-CTGACCCTACAAGATGCCAAGAG (SEQ ID NO: 329)

Reverse-ATCATGCATTGCAACATTTATTGATGGAG (SEQ ID NO: 330)

Tyrosinase: Forward-TTGGCAGATTGTCTGTAGCC (SEQ ID NO: 331)

Reverse-AGGCATTGTGCATGCTGCTT (SEQ ID NO: 332)

pMEL-17: Forward-TATTGAAAGTGCCGAGATCC (SEQ ID NO: 333)

10 Reverse-TGCAAGGACCACAGCCATC (SEQ ID NO: 334)

RTQ-PCR analysis was performed with an ABI Prism 7700 Sequence Detection System using TaqMan reagents (PE Applied Biosystems, Foster City, CA). Equal quantities of normalized RNA's were used as a template in PCR reactions for 40 cycles with GPNMB-specific primers to obtain threshold cycle (C_T) values. The following primers (5'-3') were

15 used:

Forward-TCAATGGAACCTTCAGCCTTA (SEQ ID NO: 335)

Reverse-GAAGGGGTGGGTTTTGAAG (SEQ ID NO: 336)

Probe-TET-CTCACTGTGAAAGCTGCAGCACCAG -TAMRA (SEQ ID NO: 337)

Result:

20 Our transcript expression analysis indicated that GPNMB was strongly expressed in a high percentage of human metastatic melanoma samples. Using RTQ-PCR, GPNMB was found to be highly expressed ($CT < 27.0$) in 5/7 melanoma cell lines and 5/5 melanoma clinical specimens examined (Table 50). In contrast, GPNMB was not expressed in a renal carcinoma cell line, TK-10, that was used as a negative control in our experiments.

25 Table 50: GPNMB transcript expression in human melanoma cell lines and clinical specimens

Sample Details		Expression*
30	<u>Cell lines</u>	
	UACC-62	Met. Melanoma 21.2
	M14	Met. Melanoma, amelanotic 22.2
	SK-Mel-5	Met. Melanoma, axillary node 22.9
	SK-Mel-28	Met. Melanoma, skin 24.1
35	WM-266-4	Met. Melanoma, skin 24.5
	A-375	Met. Melanoma, skin 29.0
	LOXIMV1	Met. Melanoma, amelanotic 30.9
	TK-10	Renal cell carcinoma 40.0

Clinical specimens		
5	#1	Met. Melanoma 26.6
	#2	Melanoma 26.4
	#3	Melanoma 26.9
	#4	Met. Melanoma 24.1
	#5	Met. Melanoma 25.3

* Threshold cycle (C_T) values from RTQ-PCR analysis. Met: Metastatic.

10

To extend these results, we investigated the expression of GPNMB in a panel of 17 melanoma cell lines via semi-quantitative RT-PCR (Table 51). The results show that GPNMB transcript is highly expressed in 15/17 melanoma cell lines, weakly expressed in 1/17 melanoma cell line (A-375), and not detectable in 1/17 melanoma cell line (LOXIMVI) nor in the control TK-10.

15

Table 51. RT-PCR analysis

20	Cell line	Annotation	Expression*			
			GPNMB	MART-1	Tyrosinase	pMel-17
25	M14	Met. Melanoma, amelanotic	+++	+++	+++	+++
	SK-Mel-5	Met. Melanoma, axillary node	+++	+++	+++	+++
	SK-Mel-28	Met. Melanoma, skin	+++	+++	+++	+++
	WM-266-4	Met. Melanoma, skin	+++	+++	+++	+++
	SK-Mel-2	Met. Melanoma, skin	+++	+++	+++	+++
30	UACC-257	Met. Melanoma	+++	+++	+++	+++
	A2058	Met. Melanoma, lymph node	+++	+++	+++	+++
	G361	Met. Melanoma, skin	+++	+++	+++	+++
	HT-144	Met. Melanoma, skin	+++	+++	+++	+++
	MEWO	Met. Melanoma, lymph node	+++	+++	+++	+++
35	SK-Mel-3	Met. Melanoma, Lymph node	+++	+++	+++	+++
	MALME-3M	Met. Melanoma	+++	+++	+++	+++
	UACC-62	Met. Melanoma	+++	+++	+++	-
	SK-Mel-24	Met. Melanoma, lymph node	+++	-	+++	-
	RPMI-7951	Met. Melanoma, lymph node	+++	-	+	-
40	A-375	Met. Melanoma, skin	+	-	-	-
	LOXIMVI	Met. Melanoma, amelanotic	-	-	-	-
	TK-10	Renal cell carcinoma	-	-	-	-

*RT-PCR analysis: Strongly (+++), weakly (+) or not detectable (-). Met: Metastatic.

45

Furthermore, comparing the expression of GPNMB transcript to known melanoma/melanocyte-associated gene transcripts (MART-1, tyrosinase and pMEL-17) in the melanoma cell lines (Table 51) demonstrated strong expression of MART-1, tyrosinase and pMEL-17 in 13/17, 14/17 and 12/17 melanoma cell lines, respectively. Notably, 12/17 samples co-expressed high levels of GPNMB and all three melanoma/melanocyte-associated genes. Both LOXIMVI and TK-10 cell lines, which had undetectable GPNMB

50

expression, also lacked expression of the three melanoma/melanocyte-associated genes examined.

Example 24: Growth-inhibitory Activity of CR011-vcMMAE is Dependent on GPNMB Expression

5 **Material and Methods:**

Flow cytometry: Quantitative analysis of GPNMB expression on the cell surface of cell lines was determined by flow cytometry. Approximately 1×10^6 cells were harvested, washed and incubated with a saturating amount (10 $\mu\text{g/mL}$) of either CR011 or isotype-matched control antibody in staining buffer containing PBS (pH 7.4), 4% FBS and 0.1% NaN₃ for 30 min on ice, followed by washing and staining with R-Phycoerythrin (PE)-conjugated goat-anti-human antibody (Jackson ImmunoResearch Laboratories, Inc, West Grove, PA) at 1:100 for 30 min on ice. Cells were fixed in 1% paraformaldehyde/PBS and examined on a Becton Dickinson FACSCalibur flow cytometer. Data analysis was performed with Becton Dickinson Cell Quest software version 3.3 and the geometric mean
10 fluorescence intensity ratio (GMR) was determined for each cell type.

Internalization of cell surface bound antibodies was assessed by a modified flow cytometry procedure. In brief, cell suspensions were labeled with 10 $\mu\text{g/mL}$ unconjugated or MMAE-conjugated CR011 for 30 min on ice. After washing cells, incubation was shifted to 37°C for 1 hr to allow internalization of bound antibodies. Cells that remained on ice
20 (total surface bound) or that were incubated at 37°C (internalized) were stained with PE-conjugated goat-anti-human antibody at 1:100 for 30 min to detect CR011 retained on the cell surface. Labeled cells were analyzed by flow cytometry as described above. The percentage of antibody internalized was determined using the GMRs and the following formula:

25
$$\text{Percent internalized} = \frac{\text{Total surface bound (4°C)} - \text{Total surface bound (37°C)}}{\text{Total surface bound (4°C)}} \times 100$$

Immunoprecipitation and immunoblot analysis: Cells were harvested and lysed on ice for 30 min in lysis buffer containing 1 % NP-40, 0.15 M NaCl, 0.02 M Tris-HCl, 10% glycerol, 0.01 M EDTA and complete protease inhibitor mixture (Roche Molecular
30 Biochemicals, Indianapolis, IN). Supernatants were collected and the protein concentration was determined with the BCA Protein Assay Kit (Pierce, Rockford, IL). For immunoprecipitation, 2 μg of primary antibody was added into 0.5-1 mg of total cell lysates

and incubated at 4°C for 3 hrs, followed by incubation with protein-A-agarose (Amersham Biosciences, Upsala, Sweden) on ice for 2 hrs. The agarose beads were washed in ice-cold TBST (PBS with 0.1% Tween-20). Immunoprecipitates were recovered from supernatants after boiling in Laemmli sample buffer and centrifugation.

5 For immunoblot analysis, total cell lysates (50 µg) or immunoprecipitates were resolved under reducing condition on 4-20% Tris-glycine gels (Invitrogen) and electrophoretically transferred to 0.45-µm PVDF membranes (Invitrogen). Membranes were blocked with 3% BSA (Sigma, St. Louis, MO) in TBST for 3 hrs and probed with rabbit anti-GPNMB polyclonal antibody (1:1000) for 3 hrs. Peroxidase-conjugated goat
10 anti-rabbit IgG (H+L) secondary antibody (Jackson ImmunoResearch Labs) was added and incubated for 30 min. The membranes were washed in TBST and subjected to enhanced chemiluminescence (Amersham) following the manufacturer's protocol.

Clonogenic Assays: The growth-inhibitory activity of CR011-vcMMAE was determined by clonogenic assay. Cells were plated in 96-well plates and allowed to recover
15 overnight. Unconjugated CR011, free MMAE, CR011-vcMMAE or isotype-matched vcMMAE conjugated antibody at various concentrations was added to sub-confluent cell cultures and incubated for 4 days at 37°C. The cells were then transferred into 6-well plates and allowed to form colonies. Colonies were stained with Giemsa stain (Sigma) and counted. The surviving cell fractions were calculated based upon the ratio of the treated
20 sample and the untreated control. The results were expressed as a percentage of control using GraphPad Prism Version 4 software. The IC50 was defined as the concentration resulting in a 50% reduction of colony formation compared to untreated control cultures.

Results:

To demonstrate that CR011-vcMMAE growth-inhibitory activity is dependent on
25 GPNMB expression, full-length GPNMB protein was ectopically expressed in HEK293 cells. Immunoblot (Figure 9A) and FACS (Figure 9B) analyses confirmed that GPNMB was expressed in GPNMB/plasmid transfected cells. Treatment of cells with CR011-vcMMAE, followed by clonogenic assay, demonstrated that GPNMB-expressing HEK293 cells were more sensitive to CR011-vcMMAE-mediated growth-inhibition than were
30 control cells devoid of GPNMB expression (Figure 9C).

To further verify our findings, GPNMB-expressing SK-Mel-2 cells were transfected with siRNA to specifically inhibit endogenous GPNMB expression.

Immunoblot and FACS analyses performed 2 and 4 days after transfection demonstrated that total GPNMB (Figure 10A) and surface GPNMB (Figure 10B) protein levels were significantly reduced in SK-Mel-2 cells after the transfection when compared to the control transfectants. The amount of GPNMB expression was reduced for at least 7 days after
5 transfection. Treatment of these cells with CR011-vcMMAE demonstrated that SK-Mel-2 cells were less sensitive to the growth-inhibitory activity of CR011-vcMMAE following siRNA-mediated GPNMB knockdown (Figure 10C). Taken together, these data indicate that the growth-inhibitory activity of CR011-vcMMAE required cell surface GPNMB expression.

10 **Example 25: Cell Cycle Arrest and Induction of Apoptosis by CR011-vcMMAE**

To evaluate CR011-vcMMAE's mechanism of growth inhibition, cell cycle analysis was performed.

Material and Methods:

The cell cycle effects of CR011-vcMMAE were evaluated after treating cells in
15 complete growth medium for 24 or 48 hr. Briefly, cells were pulsed at the indicated times with 30 μ M of bromodeoxyuridine (BrdU, Sigma) for 30 min, harvested, fixed and permeabilized in methanol. Nascent DNA synthesis was detected by anti-bromodeoxyuridine-FITC (BD Biosciences, San Jose, CA) staining. Total DNA content was detected using propidium iodide (PI, Sigma). For apoptosis analysis, cells were treated
20 as above and labeled with Annexin V-FITC followed by propidium iodide exclusion using the Annexin V-FITC Apoptosis Detection kit I (BD PharMingen, San Diego, CA) according to the manufacturer's protocols. Flow cytometry (as described in the previous Example) was used to assay both cell cycle and apoptosis studies.

Results:

25 GPNMB-positive SK-Mel-2 cells or negative TK-10 control cells were treated with CR011-vcMMAE for various lengths of time, followed by bromodeoxyuridine for 30 minutes to detect nascent DNA synthesis and finally, propidium iodide to detect total DNA content. DNA synthesis and cell cycle progression were determined by flow cytometry (Table 52).

Table 52. Cell cycle analysis of CR011-vcMMAE treated cells

Treatment (ng/mL)		% G ₁	% S-phase	% G ₂ /M	% Sub-G ₁
5	SK-Mel-2				
	<u>24 hour</u>				
	Untreated	55.2	30.0	9.9	0.5
	CR011 (1000)	63.6	25.2	6.4	0.5
	IgG ₂ -vcMMAE (1000)	65.9	21.8	5.8	0.8
10	CR011-vcMMAE (100)	56.0	26.9	12.4	0.2
	CR011-vcMMAE (1000)	43.7	20.0	28.5	1.1
	TK-10				
	<u>24 hour</u>				
15	Untreated	39.7	43.7	7.0	0.5
	CR011 (1000)	42.0	39.8	6.3	0.3
	IgG ₂ -vcMMAE (1000)	42.8	40.2	5.9	0.3
	CR011-vcMMAE (100)	51.1	35.1	4.5	0.7
20	CR011-vcMMAE (1000)	52.6	34.2	3.9	0.8

Cell cycle analysis was carried out by flow cytometry and the percentages of cells in each phase of cell cycle were determined by CellQuest Software (Becton Dickinson).

Exposure of GPNMB-positive cells to 1000 ng/mL CR011-vcMMAE, but not to isotype control IgG₂-vcMMAE for 24 hrs, resulted in a decreased percentage (10 %) of cells in G₁ and S-phase and an increased percentage (18.6 %) of cells in G₂/M when compared to untreated cells. In contrast, CR011-vcMMAE did not affect the cycling of GPNMB-negative cells. At 48 hr after the treatment, CR011-vcMMAE further reduced the percentage (11 %) of cells in G₁ and S-phase and increased the percentage (24 %) of cells in G₂/M.

The increase in the sub-G₁ population following CR011-vcMMAE treatment suggested the onset of apoptosis. To investigate this possibility, analysis of apoptosis using Annexin-V surface binding and loss of propidium iodide (PI) exclusion was performed. Our results demonstrated that 1000 ng/mL of CR011-vcMMAE induced apoptosis specifically in GPNMB-expressing cells as indicated by an 11 % increase in mono-stained (Annexin-V+/PI-) cells following 48 hr of CR011-vcMMAE treatment (Table 53).

Table 53. Induction of apoptosis in human melanoma cells by CR011-vcMMAE

		% AnnV ⁻ /PI ⁺	% AnnV ⁺ /PI ⁺	% AnnV ⁻ /PI ⁻	%
5	AnnV ⁺ /PI ⁻ Treatment (ng/mL) LR	UL	UR	LL	
SK-Mel-2					
48 hour					
10	Untreated	1.23	1.23	94.37	
	3.16				
	CR011 (1000)	0.36	0.45	94.45	
	4.74				
	IgG ₂ -vcMMAE (1000)	0.17	0.51	95.93	
15	3.39				
	CR011-vcMMAE (100)	0.30	0.40	89.93	
	9.37				
	CR011-vcMMAE (1000)	2.08	2.02	82.08	
	13.83				
20	TK-10				
48 hour					
	Untreated	0.54	0.66	96.92	
	1.87				
25	CR011 (1000)	0.83	0.34	98.27	
	0.55				
	IgG ₂ -vcMMAE (1000)	0.62	0.95	97.09	
	1.33				
	CR011-vcMMAE (100)	0.71	0.57	97.72	
30	1.00				
	CR011-vcMMAE (1000)	0.86	0.83	97.75	
	0.56				

35 Apoptosis analysis was carried out by flow cytometry and the percentages of cells in quadrants UL (upper left), UR (upper right), LL (lower left) and LR (lower right) were determined by CellQuest Software (Becton Dickinson). AnnV: Annexin V-FITC and PI: Propidium iodide.

40 In addition, an increase in dual-stained (Annexin-V+/PI+) cells following CR011-vcMMAE treatment indicated that the CR011 immunoconjugate enhanced cell death. Together, these results suggest that CR011-vcMMAE selectively induced G2/M cell cycle arrest followed by apoptotic cell death.

Example 26: CR011: A naked fully human IgG1 for use in melanoma therapy exploiting the mechanism of antibody-dependent cellular cytotoxicity (ADCC)

45 Fully human monoclonal antibodies (mAb)-IgG2 to CG56972/GPNMB, an antigen predominantly found on the surface of melanoma and brain tumor cells, were generated. The naked CR011 IgG2 mAb (mAb 1.15) had no effect on CG56972 expressing cells either in vitro or in vivo. Thus we examined whether isotype switching from an IgG2 to an IgG1 might enable the mAb to kill human melanoma cells through ADCC effector functions.

Briefly, to switch CR011 from an IgG2 to IgG1 antibody, double stranded DNA encoding constant region of IgG1 (allotype Gm(f)) was synthesized, and IgG2 constant region was replaced with IgG1 constant region using overlapped PCR approach. The sequences are described below:

5 CR011 mAb 1.15.1 mature heavy chain (IgG2):

QVQLQESGPGLVKPSQTLSTCTVSGGSISSFNYYWSWIRHHPGKGLEWIGYITYSG
STYSNP SLKSRVTISVDTSKNQFSLTLSSVTAADTAVYYCARGYNWNYFDYWGGQ
TLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVTVPSSNFGTQTYTCNV DHKPSNTKVDKTV ERKCCVEC
10 PPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVE
VHNAKTKPREEQFNSTFRVVS VLT VVHQDWLNGKEYKCKVSNKGLPAPIEKTISK
KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTP
PMLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK
(SEQ ID NO: 394)

15 CR011 mAb 1.15.1 mature heavy chain (IgG1):

QVQLQESGPGLVKPSQTLSTCTVSGGSISSFNYYWSWIRHHPGKGLEWIGYITYSG
STYSNP SLKSRVTISVDTSKNQFSLTLSSVTAADTAVYYCARGYNWNYFDYWGGQ
TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV EPKSCDKTH
20 TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVS VLT VLVHQDWLNGKEYKCKVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK
(SEQ ID NO: 395)

25 We first analyzed the binding properties of the IgG1 and IgG2 fully human
monoclonal antibodies on SK-MEL-2 melanoma cells that have been shown to express
CG56972 on the cell surface and bind CR011 IgG2. As shown in Figure 11, both the IgG1
and IgG2 mAbs caused comparable FACS shifts on SK-MEL-2 cells compared to isotype
control mAbs (Figure 11) indicating that both isotypes bind to CG56972/GPNMB with
30 comparable saturation densities and affinities.

We next examined whether the CR011 IgG1 mAb could induce ADCC in SK-MEL-2 cells in culture in the presence of human PBMC. Human PBMC were isolated from whole blood using a Ficoll-Plaque. Briefly, in a 50 mL tube, 15 mL of PBS was added to 20 mL of whole blood which was underlayed with 10 mL Ficoll-Plaque and the tube was
5 centrifuged at 2000 RPM. Mononuclear cells were collected from the interface and washed 3 times with PBS. The ADCC assay was carried out in a 96 well plate using a fluorescence assay for cytotoxicity from Perkin-Elmer (DELTA EuTDA Cytotoxic assay). The procedure is based on loading target cells with a fluorescence enhancing ligand (BATDA, bis (acetoxymethyl) terpyridine – dicarboxylate). The hydrophobic ligand penetrates the
10 membrane quickly. Within the cell the ester bonds are hydrolyzed to form a hydrophilic ligand (TDA, terpyridine – dicarboxylic acid) which can no longer pass through the membrane. After cytotoxicity the ligand is released and introduced to the Europium solution. The europium and the ligand form a highly fluorescent and stable chelate (EuTDA). Fluorescence intensity are recorded using excitation and emission wavelengths as $\lambda_{ex} = 340$
15 nm and $\lambda_{em} = 613$ nm, respectively.

Antibody-dependent cell-mediated cytotoxicity on SK-MEL-2 cells was assayed in the presence of PBMC and CR011 monoclonal antibody using effector: target ratios of 10, 30, 60 and 100 and various concentrations of IgG1 or IgG2 mAb against CG56972/GPNMB (2, 5, 10 μ g/200 μ l). Our data showed that between 30 to 100 fold PBMC, IgG1 mAb
20 caused cytotoxicity of SK-MEL-2 cells in a dose dependent manner (Figure 12A) whereas IgG2 mAb did not show any cytotoxicity (Figure 12B). Therefore, we conclude that CR011 IgG1 mAb to CG56972/GPNMB can kill CG56972/GPNMB expressing melanoma cells in vitro and potentially human melanoma in vivo through ADCC effector functions. CR011 IgG1 mAb can also be useful in combination with immune effector cytokines that could
25 provide some clinical benefit in metastatic melanoma such as high dose IL-2, interferon-gamma or TNF-alpha. CR011 can also be used to treat melanoma in combination with vaccine immunotherapy, immunomodulators such as MDX-010, radiation therapy and/or chemotherapy.

30 **Example 27: Treatment of Astrocytoma, Glioblastoma, Medulloblastoma and Other tumors of the CNS**

Astrocytoma/glioblastoma is a highly drug-refractory neoplasm representing significant unmet medical needs. We identified CG56972 as a human gene (also known as GPNMB) that is highly expressed in these human cancer tissues and cancer cell lines.

CG56972 is a type I transmembrane protein potentially involved in vesicular trafficking with a very restricted expression pattern in human brain. We generated fully human monoclonal antibodies against the CG56972 extracellular domain (amino acids 23-480). Our lead monoclonal antibody, designated CR011-vcMMAE was biochemically characterized and tested for therapeutic activity against cell lines derived from human brain tumors of astrocytoma, glioblastoma, medulloblastoma or neuroectodermal origin.

Transcript expression analysis demonstrated highly elevated CG56972 mRNA in brain tumors derived from astrocytoma, glioblastomas, medulloblastoma and tumors of neuroectodermal origin with restricted low expression in normal brain. CR011 bound by FACS analysis surface CG56972 on brain cancer cell lines. CR011 mAbs western blotted the predicted 100 and 120 kDa gene products. Clonogenic assays demonstrated that CR011-vcMMAE mAbs inhibited the growth of brain cancer cell lines.

Material and Methods:

Cell lines and culture conditions: All human cell lines, SK-MEL-2, XF-498, SNB-78, U-118-MG, SF-539, H79MG, D392-MG, D534-MG, SK-N-SH, U-251, SF-295, D450-MG, U87MG, SF-268, T98G, and SW-1783 were obtained from the American Type Culture Collection (Manassas, VA) or were purchased from the NCI (Bethesda, MD). Cells were maintained in DMEM or RPMI (Invitrogen, Carlsbad, CA) containing 10% FBS (Gemini Bio-Products, Woodland, CA) and penicillin-streptomycin.

Real-Time Quantitative PCR (RTQ-PCR): Total RNA was isolated using the RNeasy kit with a DNase digestion step (Qiagen Inc., Valencia). RNA samples were derived from normal human tissues obtained commercially (Clontech, Palo Alto, CA; Invitrogen, Carlsbad, CA) or cell lines grown according to specifications. RNAs were harvested and PCR was performed as previously described (Shimkets RA et. al. Nat Biotechnol., 1999. 17-8: 798-803) using TaqMan® reagents (PE Applied Biosystems, Foster City, CA). RNAs were normalized utilizing human β -actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) TaqMan® probes according to the manufacturer's instructions. Equal quantities of normalized RNA were used as templates in PCR reactions with CG56972-specific reagents to obtain threshold cycle (CT) values. For graphic representation, CT numbers were converted to relative expression, relative to the sample exhibiting the highest level of expression. RTQ-PCR analysis was performed with an ABI

Prism 7700 Sequence Detection System using TaqMan reagents (PE Applied Biosystems, Foster City, CA). The following primers (5'-3') were used:

- Forward-TCAATGGAACCTTCAGCCTTA (SEQ ID NO: 338)
 Reverse-GAAGGGGTGGGTTTTGAAG (SEQ ID NO: 339)
 5 Probe-TET-CTCACTGTGAAAGCTGCAGCACCAG-TAMRA (SEQ ID NO: 340)

CuraChip™: Tissues were lysed in Trizol. Biotin-labeled cDNA was made by using 15 mg of total RNA with poly(T) primers. Gene expression was evaluated by hybridization to the proprietary CuraChip microarray (CuraGen, New Haven, CT) of 11,000 oligonucleotide probes. Slides were hybridized for 15 h at 30°C with constant rotation,
 10 washed for 30 min at room temperature (RT), incubated in streptavidin solution (4°C, 30min), washed three times for 15 min at RT, incubated in Cy3-conjugated detection buffer (4°C, 30 min), and washed three times for 15 min at RT. Slides were scanned (GMS 418 Scanner, Genetic Microsystems, Woburn, MA) and analyzed by using IMAGENE software (BioDiscovery, Marina Del Rey, CA). Data was subjected to 90th percentile normalization,
 15 and expression of the CG56972 gene was analyzed in comparison to that of the housekeeping gene GAPDH. The oligonucleotide sequence used to detect CG56972 is 5'-TGATCAGTAAGGATTCACCTCTGTTTGTA (SEQ ID NO: 341). The oligonucleotide sequence used to detect GAPDH is 5'-ACCTTGTCATGTACCATCAATAAAGTACCC (SEQ ID NO: 342), corresponding to bp 1243-1272 of the GAPDH transcript (accession no.
 20 NM_002046).

Flow Cytometry: Quantitative analysis of CG56972 expression on the surface of cell lines was determined by flow cytometry (FACS). Approximately 1×10^6 cells were harvested, washed and incubated with a saturating amount (10 µg/mL) of either CR011 or isotype-matched control antibody in staining buffer containing PBS (pH 7.4), 4% FBS and
 25 0.1% NaN₃ for 30 min on ice, followed by washing and staining with R-Phycoerythrin (PE)-conjugated goat-anti-human antibody (Jackson ImmunoResearch Laboratories, Inc, West Grove, PA) at 1:100 for 30 min on ice. Cells were fixed in 1% paraformaldehyde/PBS and examined on a Becton Dickinson FACSCalibur flow cytometer. Data analysis was performed with Becton Dickinson Cell Quest software version 3.3 and the geometric mean
 30 fluorescence intensity ratio (GMR) was determined for each cell type.

Immunoblot analysis: SK-MEL-2, XF-498, SNB-78, U-118-MG, SF-539, H79MG, D392-MG, D534-MG, SK-N-SH, U-251, SF-295, D450-MG, U87MG, SF-268, T98G, and SW-1783 cells were harvested and lysed on ice for 30 min in lysis buffer containing 1 %

NP-40, 0.15 M NaCl, 0.02 M Tris-HCl, 10% glycerol, 0.01 M EDTA and complete protease inhibitor mixture (Roche Molecular Biochemicals, Indianapolis, IN). Supernatants were collected and the protein concentration was determined with the BCA Protein Assay Kit (Pierce, Rockford, IL). For immunoblot analysis, 40 μ l of total cell lysate from one well of
5 confluent cells harvested from a 6 well Falcon tissue culture dish were boiled in Laemmli sample buffer, centrifuged and resolved under reducing condition on 4-20% Tris-glycine gels (Invitrogen). Gels were electrophoretically transferred to 0.45- μ m PVDF membranes (Invitrogen). Membranes were blocked with 3% BSA (Sigma, St. Louis, MO) in TBST for 3 hrs and probed with goat anti-GPNMB polyclonal IgG (R & D Systems; 1 μ g/mL, total 10
10 μ g)) for 3 hrs. Peroxidase-conjugated anti-goat secondary antibody (Jackson ImmunoResearch Labs) was added and incubated for 30 min. The membranes were washed in TBST and subjected to enhanced chemiluminescence (Amersham) following the manufacturer's protocol.

Clonogenic assays: The growth-inhibitory activity of CR011-vcMMAE was
15 determined by clonogenic assay. Cells were plated in 96-well plates and allowed to recover overnight. CR011-vcMMAE or isotype-matched monoclonal antibody at various concentrations was added to sub-confluent cell cultures and incubated for 4 days at 37°C. The cells were then transferred into 6-well plates and allowed to form colonies. Colonies
20 were stained with Giemsa stain (Sigma) and counted. The surviving cell fractions were calculated based upon the ratio of the treated sample and the untreated control. The results were expressed as a percentage of control using GraphPad Prism Version 4 software. The IC50 was defined as the concentration resulting in a 50% reduction of colony formation compared to untreated control cultures.

Results:

- 25 1. CG56972 transcript expression in human astrocytoma, glioblastoma, medulloblastoma and tumors of neuroectodermal origin.

We examined the expression of CG56972 transcripts in human cancer cell lines and tissues (Figures 13 A & B). Our transcript expression analysis indicated that CG56972 was strongly expressed in all (15/15) human brain cancer cell lines tested (Figure 13A)
30 Using RTQ-PCR, CG56972 was found to be expressed in cells of mixed glioblastoma/astrocytoma origin, glioblastoma/gliomas, astrocytomas and metastatic neuroblastomas. The majority of brain or CNS tumor cell lines showed high level

expression with CTs<27. Of note, CG56972 was found to be highly expressed (CT<27.0) in XF-498, U-118-MG, SNB-78, and SF-539 cells. As shown in Figure 13B, CG56972 was also expressed at high levels in 4/5 glioma human biopsies and 1/4 medulloblastoma human biopsies. Using microarray analysis from an in house chip containing a large panel of human genes (Figure 13C), CG56972 was found to be highly expressed in 5/9 brain cancers of astrocytoma or glioblastoma origin as well as 4/9 oligodendrogliomas. Our analysis of these tumor expression profiles showed that CG56972 message was detected to a much lesser degree in normal brain tissues. These data are also consistent with our immunohistochemical data that demonstrated the lack of CR011 staining in normal human brain including neurons and glial cells. Taken together, these data demonstrate that the CG56972 transcript is expressed at highly elevated quantities in brain cancer and oligodendroglioma cell lines and specimens isolated from human tumors.

2. Generation of fully human CR011 monoclonal antibodies to CG56972/GPNMB

The CG56972 protein is predicted to be a type I transmembrane glycoprotein. The highly elevated expression of CG56972 transcripts and the potential cell surface localization of this protein in human cancer samples encouraged us to generate monoclonal antibodies (mAbs) as a potential cancer therapeutic. Therefore, we cloned the human CG56972 extracellular domain (ECD; aa 23-480). Sequencing of the cloned cDNA revealed the presence of an in-frame 36-nt insertion, likely due to alternative splicing at the exon 6/7 boundary, which added an additional 12-aa (ATTLKSYDSNTP) (SEQ ID NO: 343) after residue 339 of the published GPNMB protein sequence. We verified the authenticity of 36-nt insertion via RT-PCR. The cDNA was next expressed in human HEK293 cells. The resultant protein was harvested, purified from the conditioned media and used as an immunogen to generate fully human mAbs against CG56972-ECD. Following immunization of XenoMouse®, mAbs that specifically recognized the CG56972-ECD protein via ELISA were generated. Our lead mAb, designated 1.15 or CR011 against purified CG56972-ECD, exhibiting a Kd of 52 nM against purified CG56972-ECD protein, was selected for in depth characterization and will be the focus of the remainder of this example.

3. CR011 mAb 1.15 detection of CG56972 protein expression in human brain cancers

We further used CR011 monoclonal antibodies to examine the surface expression of CG56972 protein on a variety of brain cancer cell lines by flow cytometry

(Figure 14 & Table 54). FACS analysis demonstrated that the brain cancer cell lines XF-498, U-118-MG, SNB-78 and SF-539; all positive for CG56972 transcript expression exhibited surface staining with CR011 monoclonal antibodies of at least 1.5-fold above isotype control mAb background (Figure 14). The cell line SF-268, that was the most weakly positive (CT>32) for the CG56972 transcript expression (Figure 13C & Table 54) showed minimal surface staining as expected of around 1.5-fold above control mAb background. Our SK-MEL-2 melanoma cell line that is our positive control for CG56972 expression showed strong cell surface staining.

To investigate CG56972 protein expression in our panel of brain cancer cell lines, total cell lysates were harvest, resolved by SDS-PAGE, transferred to membrane filters and subjected to immunoblot analysis with a CG56972 polyclonal antibody. As shown in Figure 15, the CG56972 polyclonal antibody detected two protein species that are differential glycosylation products of CG56972 of approximately 100 and 120 kDa from various brain cancer cell lines that have been shown to express CG56972 transcripts (Figure 13A). CG56972 protein was highly expressed in XF-498, SNB-78 and H79-MG and SF-539 cells. Both p100 and p120 species were detected to a lesser extent in U-118-MG, U251, D534-MG and D450-MG. Little or no CG56972 protein was detected in weakly expressing CG56972 transcript cell line, SF-268. An isotype-matched control IgG2 antibody did not immunoprecipitate CG56972 from any of the cell lines examined. All of these data are consistent with the cell surface expression of the CG56972 protein of the predicted molecule weights in brain cancer.

4. In vitro growth-inhibition of astrocytoma/glioblastoma cell lines with CR011-vcMMAE.

CG56972 possesses a very restricted human tissue expression pattern. In preliminary studies, CR011 did not inhibit the growth of CG56972-expressing cancer cell lines when used directly (data not shown). Since CG56972 is a cell surface molecule on brain cancers and melanoma, and since CR011 was internalized following incubation with CG56972-expressing cells, we evaluated whether CR011 would inhibit the growth of cancer cells when combined with a protein synthesis inhibitor (saporin)-conjugated secondary antibody. Our results indicated that CR011 could specifically inhibit the growth of CG56972-expressing cancer cells (data not shown). Thus, we conjugated CR011 directly to the cytotoxic drug monomethyl aurostatin E (MMAE) through a highly stable, but

intracellular protease cleavable valine-citrulline (vc) linker. The resulting antibody-drug conjugate was named CR011-vcMMAE.

To investigate whether CR011-vcMMAE specifically inhibited the growth of brain cancer cells, clonogenic assays were performed to assess cell viability after CR011-vcMMAE treatment. As shown in Figure 16 and Table 54, our results indicated that CG56972-expressing cells were sensitive to growth-inhibition induced by CR011-vcMMAE, but not cells that poorly expressed this antigen (see SF-268 and LOXIMVT) at concentrations of vcMMAE less than 3 $\mu\text{g/mL}$. Strikingly, CR011-vcMMAE possessed IC50s of approximately 215, 450, 1250, and 1050 ng/mL on XF-498, SNB-78, U-118-MG and SF-539 cells (Figure 16 and Table 54). In these experiments, IC50s correlated with cell surface density as measured by FACS analysis. In contrast, conjugated control human IgG2 antibody-vcMMAE failed to inhibit the growth of any of the cell lines examined at concentrations up to 3 $\mu\text{g/mL}$ (Table 54) with IC50s exceeding 1.5 or 4.5 $\mu\text{g/mL}$ (Table 54).

Table 54. Summary of RTQ PCR, FACS and in vitro growth inhibition of human cancer cell lines with CR011-mAbs

Cell Line	Description	CT Values	CR011 Fold Shift	CR011-AE IC50 (ng/ml)	IgG2-AE IC50 (ng/ml)
SK-MEL-2	melanoma	ND	13.1, 21.4, 17.8	303	>1500
XF-498	glioblastoma	+++	10, 9.5	216	>1500
SNB-78	astrocytoma	+++	8.6	449	>1500
U-118-MG	glioblastoma/astrocytoma	+++	7.4	1254	>1500
SF-539	glioblastoma	+	5.4	1030	>4500
H79MG	glioblastoma/astrocytoma	ND	4.7, 3.9	ND	ND
D392-MG	glioblastoma	ND	3.1	ND	ND
D534-MG	glioblastoma	ND	2.3	ND	ND
SK-N-SH	neuroblastoma	+	2	ND	ND
U-251	glioblastoma	+++	1.9	ND	ND
SF-295	glioblastoma	++	1.8	ND	ND
D450-MG	glioblastoma	ND	1.6	ND	ND
U87MG	glioblastoma/astrocytoma	++	1.5	ND	ND
SF-268	glioblastoma/astrocytoma	+	1.5	>1500	>4500
T98G	glioblastoma	+	1.3	ND	ND
SW 1783	astrocytoma	+	1.1	ND	ND

^a CR011vcMMAE (mAb 1.15): CT values were determined by RTQ PCR as described in Materials and Methods. Geometric Mean ratios (GMR) were determined by flow cytometric analysis. Antibody-Drug Cytotoxicity (ADC) or cell killing was determined by clonogenic assay as described. ^b IC₅₀ value is the mean and SD of a representative clonogenic assay with each experiment performed in triplicate wells. ND: Not done.

Conclusion:

These data indicate that CR011-vcMMAE can be a highly potent and selective agent for the treatment of astrocytoma/glioblastoma and their metastasis as well as brain tumors of medulloblastoma and neuroectodermal origin. CR011-vcMMAE can also be useful for the treatment of melanoma metastasis to brain and other brain neoplasms such as neoplastic meningitis.

Example 28: Engineered Antibodies Derived from CR011

The CR011 bi-scFv's (see Figure 17) of this work were designed to bind to a CD3 epitope of the T cell receptor on cytotoxic human T lymphocytes and, at the same time, to target diseased cells expressing GPNMB, with the net result of facilitating the lysis or destruction of the diseased cells.

The V_L and V_H domains of mAb CR011, clone 1.15 were used in the construction of 3 CR011 based engineered antibodies:

- (1) CR011 single chain antibody (CR011 scFv)
- 15 (2) CR011 x anti-CD3 bispecific single chain antibody (bi-scFv), Linker set L4-L2-L4
- (3) CR011 x anti-CD3 bispecific single chain antibody (bi-scFv), Linker set L4-L4-L4

The components of the CR011 scFv protein were: Signal Peptide-V_L (CR011)-Linker 4-V_H (CR011)-Flag Tag. The components of the CR011 x anti-CD3 bi-scFv (Linker set L4-L2-L4) protein were: Signal Peptide-V_L (CR011)-Linker 4-V_H (CR011)-Linker 2-V_H (anti-CD3)-Linker 4-V_L (anti-CD3)-Flag Tag. The components of the CR011 x anti-CD3 bi-scFv (Linker set L4-L4-L4) protein were: Signal Peptide-V_L (CR011)-Linker 4-V_H (CR011)-Linker 4-V_H (anti-CD3)-Linker 4-V_L (anti-CD3)-Flag Tag.

The various DNA components outlined above were used to generate the three CR011 engineered antibody products. The DNA components were synthesized by Blue Heron and cloned into commercially available plasmid vectors by methods familiar to those skilled in the art. These plasmids were then used in PCRs to combine the components, indicated in the 3 examples above, to generate engineered antibody inserts for expression vectors. In the host expression system examples practicing this invention described below, we have used CHOK1 mammalian cells for the CR011 expression vectors, but expression is not limited to these cells; it will be recognized by those skilled in the art that the CR011 engineered antibodies of this invention can be expressed using other vectors, systems and

cells, including but not limited to: pET vectors, inducible and constitutive promoters, and hosts may include *E. coli*, *Bacillus* species, yeast, including *Pichia pastoris* or insect cells. Other expression hosts can also include various plant species and transgenic animals such as goats.

- 5 *SP (Signal Peptide)*: We incorporated a signal peptide in our constructs in order to express products that will be secreted. The signal peptide which was utilized for expression from CHO cells was derived from an immunoglobulin light chain leader peptide (Jirik *et al.*, 1986), or from the CR002 antibody (CuraGen).

- 10 *Order of the bi-scFv components*: The order of the antibody variable domains was fixed in both bi-scFv constructs as follows: V_L1-L-V_H1-L-V_H2-L-V_L2. Each of the 4 V domains was linked by a linker segment, L. V_L1 and V_H1 represent the immunoglobulin light and heavy chain variable domains respectively of CR011, and V_H2 and V_L2 represent the immunoglobulin heavy and light chain variable domains respectively of an anti-CD3 antibody that was used for both bi-scFv constructs. Other orders of the V domains can also
15 be used for the 2 scFv components, as recognized by those skilled in the art, and the products evaluated for biological activity.

Tag: We used the 8 amino acid Flag tag at the C-terminus of the CR011 engineered antibodies to facilitate detection and purification of the products (Hickman *et al.*, 2000).

- 20 *Anti-CD3 scFv*: The sequences of the V_L and V_H components of the anti-CD3 antibody used to generate the bi-scFv constructs may be found in the NCBI database under accession number CAE85148 (Lutterbuese *et al.*)

- 25 *Linkers used in constructs*: The sequence of L2, a short 5 amino acid linker that links the 2 monomer scFv components together in CR011 x anti-CD3 bi-scFv (L4-L2-L4 linker set) is G₄S (Mack *et al.*, 1995). L4 is a 25 amino acid linker based on the 205C linker (Denzin *et al.*, 1991): LSADDAKKDAKKDDAKKDDAKKDL (SEQ ID NO: 344) and is used in both of the CR011 bi-scFv species to link the CR011 V_L and V_H and the anti-CD3 V_H and V_L. In the case of the CR011 x anti-CD3 bi-scFv (L4-L4-L4 linker set), L4 is also used to link the 2 monomer scFv components together. For the CR011 scFv, the L4 linker was used to link the two variable domains together.

- 30 1. DNA plasmid constructs for expression of CR011 scFv and CR011 X anti-CD3 bi-scFv species

- CR011 scFv Flag tag: The PCR amplification product for generating the expression construct for CR011 scFv was generated from a synthetic DNA template (Blue Heron) using the F1/ R1 primers followed by nested PCR with the F1 nested/ R1 primer pair (Table 55) and Pfu Turbo DNA polymerase (Stratagene, cat# 600322), as per the manufacturer's directions.
- 5 A Sal I/EcoR I PCR fragment coding for the CR011 scFv cassette was cloned into the corresponding restriction sites of the pCTN vector (CuraGen Corporation, mammalian expression vector) using the Fast-Link DNA Ligation kit (Epicentre, cat# LK11025).

Table 55

Name	Sequence
F1 Primer	5' – TCTCTTCCTCCTGCTACTCTGGCTCCCAGATACCACCGGTGAAATAGTGATGACGCA GTC (SEQ ID NO: 345)
R1 Primer	5' – CCGGAATTCTTACTATTTGTCATCATCGTCCTTATAATCGCTAGCTGAGGAGACGGT (SEQ ID NO: 346)
F1 Nested Primer	5' – ACGCGTCGACCCACCATGGAAGCCCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGG CTC (SEQ ID NO: 347)
F2 Primer	5' – TCTCTTCCTCCTGCTACTCTGGCTCCCAGATACCACCGGTGAAATAGTGATGACGCA GTC (SEQ ID NO: 348)
R2 Primer	5' – CCGGAATTCTTACTATTTGTCATCATCGTCCTTATAATCGCTAGCTTTCAGCTCCAG (SEQ ID NO: 349)
F2 Nested Primer	5' – ACGCGTCGACCCACCATGGAAGCCCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGG CTC (SEQ ID NO: 350)
F3 Primer	5' – ACTCTGGCTCCCAGATACCACCGGAGAAATAGTGATGACGCAGTCTCCAGCCACC (SEQ ID NO: 351)
R3 Primer	5' – CCGCTCGAGCTATTTGTCATCATCGTCCTTATAATCTTTCAGCTCCAGCTT (SEQ ID NO: 352)
F3 Nested Primer	5' – TCTTCGCGACCACCATGGAACCCCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGC TCCCAGATACCACCGGA (SEQ ID NO: 353)

- 10 CR011 x anti-CD3 bi-scFv (L4-L2-L4) linker set Flag tag: The PCR amplification product for the CR011 x anti-CD3 bi-scFv having the (L4-L2-L4) linker set, was generated from a synthetic DNA template (Blue Heron) using the F2/ R2 primers followed by nested PCR with the F2 nested/ R2 primer pair (see Table 55 for sequences of oligonucleotides) and Pfu Turbo DNA polymerase (Stratagene, cat# 600322), as per the manufacturer's directions.
- 15 The Sal I/EcoR I PCR fragment having the coding sequence for the CR011 x anti-CD3 (L4-

L2-L4) bi-scFv was cloned into the corresponding sites of the pCTN vector using Fast-Link DNA Ligation kit (Epicentre, cat# LK11025).

CR011 x anti-CD3 bi-scFv (L4-L4-L4) linker set Flag tag: The PCR amplification product for the CR011 x anti-CD3 bi-scFv having the (L4-L4-L4) linker set, was generated from a synthetic DNA template (Blue Heron) using the F3/ R3 primers followed by nested PCR with the F3 nested/ R3 primer pair (Table 55) and Pfu Turbo DNA polymerase (Stratagene, cat# 600322), as per the manufacturer's directions. The Nru I/Xho I PCR fragment having the coding sequence for the CR011 x anti-CD3 (L4-L4-L4 linker set) bi-scFv was cloned into the corresponding sites of the pEE14.4FL2 expression vector (Lonza Biologics plc, 228 Bath Road, Slough, Berkshire SL1 4Dx, UK) using the Fast-Link DNA Ligation kit (Epicentre, cat# LK11025).

The DNA sequences of the above 3 expression construct inserts were verified by sequencing both strands of the relevant DNA products.

2. Protein Production of the CR011 Engineered Antibodies in CHOK1 cells

Adherent Chinese Hamster Ovary (CHOK1) cells (ATCC catalog# CCL-61) were grown in DMEM media (Invitrogen, cat# 10564-011) supplemented with 10% fetal bovine serum (Gemini, cat#100106), GS supplement (JRH Biosciences, cat# 58672-100M) and 50 mg/L gentamicin (Invitrogen, cat# 15750078).

CHOK1 cells were transfected with FuGENE 6 reagent (Roche, cat # 1815075) according to the manufacturer's directions. Expression and secretion was verified by Western blotting performed ca. 48 hours after the transfections. Selection of stable secreted CR011 scFv and CR011 x anti-CD3 bi-scFv (L4-L2-L4 linker set) lines were performed in selection media A (Table 56), while selection of a stable secreted CR011 x anti-CD3 bi-scFv (L4-L4-L4 linker set) line was performed in selection media B (Table 57).

Table 56

CHOK1 (Adherent) Selection Media A	Vendor	Item No.	Description
DMEM-glutamine free	JRH Biosciences	51435- 1000M	Glutamine-Free Media for GS System(TM) (DMEM/High Modified)
10% dialyzed FBS (heat inactivated 56°C for 30 minutes)	JRH Biosciences	12117- 500M	Fetal Bovine Serum, Dialyzed (500mL)
1X GS Supplement	JRH	58672-	GS Supplement (50X)

	Biosciences	100M	
50 mg/L gentamicin	Invitrogen	15750078	Gentamicin Reagent Solution (50 mg/mL), liquid
1 mg/mL G418	Invitrogen	10131027	Geneticin (G418)

Table 57

CHOK1 (Adherent) Selection Media B	Vendor	Item No.	Description
DMEM-glutamine free	JRH Biosciences	51435-1000M	Glutamine-Free Media for GS System(TM) (DMEM/High Modified)
10% dialyzed FBS (heat inactivated 56°C for 30 minutes)	JRH Biosciences	12117-500M	Fetal Bovine Serum, Dialyzed (500mL)
1X GS Supplement	JRH Biosciences	58672-100M	GS Supplement (50X)
50 mg/L gentamicin	Invitrogen	15750078	Gentamicin Reagent Solution (50 mg/mL), liquid
25 μ M MSX	Sigma	M 5379	L-Methionine sulfoximine (MSX)

- In each case, 8 out of 96 CR011 scFv and CR011 x anti-CD3 (L4-L2-L4 linker set) bi-scFv CHOK1 clones that were secreting products were expanded and archived. The best stable clones secreting products in each case were adapted to suspension culture in shake flasks with selection media C (Table 58) at 37°C and 5% CO₂. Protein production for CR011scFv and CR011xCD3 (L4-L2-L4 linker set) bi-scFv was carried out in 4 L of selection media D (Table 59) at 30°C and 5% CO₂.

10 Table 58

CHOK1 Large Scale (Suspension) Selection Media C	Vendor	Item number	Description
Ex-Cell 302	JRH Biosciences	14324-1000M	Ex-Cell 302 CHO Serum-free medium without L-glutamine (1000mL)
5% FBS	JRH Biosciences	12117-500M	Fetal Bovine Serum, Dialyzed (500mL)
GS Supplement	JRH Biosciences	58672-100M	GS Supplement (50X)
HT Supplement	Invitrogen	11067030	HT Supplement (100X)
1mg/mL G418	Invitrogen	10131027	Geneticin (G418)

Table 59

CHOK1 Large Scale (Suspension) Selection Media D	Vendor	Item number	Description
Ex-Cell 302 + Ex- Cell CD CHO (1:1)	JRH Biosciences	14324-1000M	Ex-Cell 302 CHO Serum-free medium without L-glutamine (1000mL)
	JRH Biosciences	14360-1000M	CD CHO Medium (1000mL)
5% FBS	JRH Biosciences	12117-500M	Fetal Bovine Serum, Dialyzed (500mL)
GS Supplement	JRH Biosciences	58672-100M	GS Supplement (50X)
HT Supplement	Invitrogen	11067030	HT Supplement (100X)
1mg/mL G418	Invitrogen	10131027	Geneticin (G418)

Only one out of two hundred CR011 x anti-CD3 (L4-L4-L4 linker set) bi-scFv
CHOK1 clones was found to produce a secreted product; this clone was expanded and
5 archived. Protein production for the CR011 x anti-CD3 (L4-L4-L4 linker set) clone was
carried out using a cell factory apparatus (Nunc, cat#164327), in selection media B (Table
57), 1 mM sodium butyrate (Sigma, cat# B5887) at 37°C and 10% CO₂.

3. Protein Purification of the CR011 Engineered Antibodies

Protein purification for the CR011 scFv Flag and CR011 x anti-CD3 (L4-L2-L4 linker
10 set) bi-scFv Flag was accomplished in three chromatography steps, including affinity, ion
exchange and size exclusion chromatographies. For the purification of CR011 x anti-CD3
(L4-L4-L4 linker set) bi-scFv Flag protein, affinity and size exclusion chromatographies were
used.

Affinity chromatography was performed using anti-FLAG M2 affinity gel (Sigma,
15 cat# A2220-25 mL) as per the manufacturer's instructions on a BioCAD 700E instrument
(Applied Biosystems). Ion exchange chromatography was performed on a MonoQ 5/50 GL
column (Amersham, cat# 17-5166-01) using 20 mM Tris-HCl pH7.5 as equilibration buffer
and a gradient elution with 0 – 1 M NaCl. Size exclusion chromatography was performed
using a Superdex 75/10/300 GL column (Amersham, cat#17-5174-01) following the
20 manufacturer's protocols on BioCAD 700E (Applied Biosystems) liquid chromatography
instrument.

The approximate yields from 1 L conditioned CHOK1 media were:

(1) CR011 scFv: 1.0 mg

(2) CR011 x anti-CD3 (L4-L2-L4 linker set) bi-scFv: 0.5 mg

(3) CR011 x anti-CD3 (L4-L4-L4 linker set) bi-scFv: 1.5 mg

The N-terminal amino acid sequence of the purified proteins was determined by
 5 Edman degradation, using methods known to those skilled in the art. The sequence of the first
 five amino acids was: E I V M T in each case (the mature N-terminus of the CR011 V_L
 protein), indicating that accurate processing by signal peptidase had occurred to give a
 soluble, secreted product of the predicted sequence and size.

The DNA and amino acid sequences of the 3 CR011 engineered products are given
 10 below.

SEQ ID for CR011 scFv - (V_L-L4-V_H) Flag. The Signal peptide of Human kappa light chain
 was used as described in Kabat *et al.* 45 CLL-CL). There was a FLAG tag included at the
 C-terminus. The Kozak sequence CCACC was included in the 5' PCR primer.

ATGGAAGCCCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCCCAGATACCACCGGTGAAAT
 15 AGTGATGACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCA
 GGGCCAGTCAGAGTGTGACAACAACCTAGTCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAG
 GCTCCTCATCTATGGTGCATCCACCAGGGCCACTGGTATCCCAGCCAGGTTCACTGGCAGTGGGT
 CTGGGACAGAGTTCACTCTCACCATCAGTAGTCTGCAGTCTGAAGATTTTGCAGTTTACTGTCT
 AGCAGTATAATAACTGGCCTCCGTGGACGTTCCGCCAAGGGACCAAGGTGGAATCAAACCTTC
 20 CGCGGACGATGCGAAAAAGGATGCTGCGAAGAAAGATGACGCTAAGAAAGACGATGCTAAAAA
 GGACCTGCAGGTGCAGCTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCACAGACCCTGTCC
 CTCACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTTTAACTACTGGAGCTGGATCCGCCAC
 CACCCAGGGAAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTACTCCAACC
 CGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTCTCCCTGACGCTG
 25 AGCTCTGTGACTGCCGCGGACACGGCCGTGATTACTGTGCGAGAGGGTATAACTGGAACCTACT
 TGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCTAGCGATTATAAGGACGATGAT
 GACAAATAGTAA (SEQ ID NO: 354)

MEAPAQLLFLLLLWLPDTTGEIVMTQSPATLSVSPGERATLSCRASQSVDDNNLVWYQQKPGQAPRLL
 IYGASTRATGIPARFSGSGTEFTLTISLQSEDAVYYCQYNNWPPWTFGQGTKVEIKLSADDAK
 30 KDAAKKDDAKKDDAKDLQVQLQESGPGLVKPSQTLSTCTVSGGSISFNYYWSWIRHHHPGKGLE
 WIGYIYYSGSTYNSPLKSRVTISVDTSKNQFSLTSSVTAADTAVYYCARGYNWNYFDYWGQGLV
 TVSSASDYKDDDDK (SEQ ID NO: 355)

SEQ ID for CR011 x anti-CD3 (L4-L2-L4 linker set) bi-scFv - The Signal peptide of
 Human kappa light chain was used as described in Kabat *et al.* 45 CLL-CL). There was a
 35 FLAG tag included at the C-terminus.

ATGGAAGCCCCAGCGCAGCTTCTCTTCTCCTGCTACTCTGGCTCCCAGATACCACCGGTGAAAT
 AGTGATGACGCAGTCTCCAGCCACCTGTCTGTGTCTCCAGGGGAAAGAGCCACCTCTCCTGCA
 GGGCCAGTCAGAGTGTGACAACAACCTTAGTCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAG
 GCTCCTCATCTATGGTGCATCCACCAGGGCCACTGGTATCCCAGCCAGGTTCAAGTGGCAGTGGGT
 5 CTGGGACAGAGTTCAGTCTCACCATCAGTAGTCTGCAGTCTGAAGATTTTGCAGTTTATTACTGTC
 AGCAGTATAATAACTGGCCTCCGTGGACGTTCCGGCCAAGGGACCAAGGTGGAAATCAAACCTTC
 CGCGGACGATGCGAAAAAGGATGCTGCGAAGAAAGATGACGCTAAGAAAGACGATGCTAAAAA
 GGACCTGCAGGTGCAGCTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCACAGACCTGTCC
 CTCACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTTTTAATTACTACTGGAGCTGGATCCGCCAC
 10 CACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTACTCCAACC
 CGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTCTCCCTGACGCTG
 AGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGGGTATAACTGGAACCTACTT
 TGACTACTGGGGCCAGGGAACCTGGTCACCGTCTCCTCAGGAGGTGGTGGATCCGATATCAAA
 CTGCAGCAGTCAGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCCTGCAAGACTT
 15 CTGGCTACACCTTTACTAGGTACACGATGCACTGGGTAACAGAGGCCTGGACAGGGTCTGGA
 ATGGATTGGATACATTAATCCTAGCCGTGGTTATACTAATTACAATCAGAAGTTCAAGGACAAGG
 CCACATTGACTACAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGA
 GGACTCTGCAGTCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGGGCC
 AAGGCACCACTCTCACAGTCTCCTCACTTTCCGCGGACGATGCGAAAAAGGATGCTGCGAAGAA
 20 AGATGACGCTAAGAAAGACGATGCTAAAAAGGACCTGGACATTACAGCTGACCCAGTCTCCAGCA
 ATCATGTCTGCATCTCCAGGGGAGAAGGTCACCATGACCTGCAGAGCCAGTTCAAGTGTAAGTT
 ACATGAACTGGTACCAGCAGAAAGTCAGGCACCTCCCCAAAAGATGGATTTATGACACATCCAA
 AGTGGCTTCTGGAGTCCCTTATCGCTTCAGTGGCAGTGGGTCTGGGACCTCATACTCTCTCAAA
 TCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCT
 25 CACGTTCCGTGCTGGGACCAAGCTGGAGCTGAAAGCTAGCGATTATAAGGACGATGATGACAAA
 TAGTAA (SEQ ID NO: 356)

MEAPAQLFLLLLWLPDTTGEIVMTQSPATLSVSPGERATLSCRASQSVDDNNLVWYQQKPGQAPRLL
 IYGASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYNNWPPWTFGQGTKVEIKLSADDAK
 KDAAKKDDAKKDDAKKDLQVQLQESGPGLVKPSQTLSTCTVSGGSISSFNYYWSWIRHHPGKGLE
 30 WIGYIYYSGSTYSNPSLKSRTISVDTSKNQFSLTSSVTAADTAVYYCARGYNWNYFDYWGQGTLLV
 TVSSGGGGSDIKLQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQLEWIGYINPSRGY
 TNYNQKFKDKATLTDDKSSSTAYMQLSSLTSEDSAVYYCARYDDHYCLDYWGQGTLLTVSSLSAD
 DAKKDAAKKDDAKKDDAKKDLQVQLQESGPGLVKPSQTLSTCTVSGGSISSFNYYWSWIRHHPGKGLE
 RWIYDTSKVASGVYPYRFSGSGSGTSYSLTISSMEEAEDAATYYCQQWSSNPLTFGAGTKLELKASDYK
 35 DDDDK (SEQ ID NO: 357)

SEQ ID for CR011 x anti-CD3 (L4-L4-L4 linker set) bi-scFv – The Signal peptide of
 CR002 was used. There was a FLAG tag included at the C-terminus.

ATGGAAACCCAGCGCAGCTTCTCTTCTCCTGCTACTCTGGCTCCCAGATACCACCGGAGAAAT
 AGTGATGACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCA
 GGGCCAGTCAGAGTGTGACAACAACCTTAGTCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAG
 GCTCCTCATCTATGGTGCATCCACCAGGGCCACTGGTATCCCAGCCAGGTTCA GTGGCAGTGGT
 5 CTGGGACAGAGTTCACTCTCACCATCAGTAGTCTGCAGTCTGAAGATTTTGCAGTTTATTACTGTC
 AGCAGTATAATAACTGGCCTCCGTGGACGTTCCGCCAAGGGACCAAGGTGGAAATCAAACCTTTC
 CGCGGACGATGCGAAAAAGGATGCTGCGAAGAAAGATGACGCTAAGAAAGACGATGCTAAAAA
 GGACCTGCAGGTGCAGCTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCAAGACCCCTGTCC
 CTCACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTTTAAATTACTACTGGAGCTGGATCCGCCAC
 10 CACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTACTCCAACC
 CGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTCTCCCTGACGCTG
 AGCTCTGTGACTGCCCGGACACGGCCGTGTATTACTGTGCGAGAGGGTATAACTGGAACCTACTT
 TGACTACTGGGGCCAGGGAACCCCTGGTCACCGTCTCCTCATTATCAGCGGATGACGCCAAGAAA
 GACGCAGCCAAAAAGGACGATGCAAAGAAGGATGACGCAAAGAAAGATTTAGATATCAAACCTG
 15 CAGCAGTCAGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCCTGCAAGACTTCTG
 GCTACACCTTTACTAGGTACACGATGCACTGGGTAACAGAGGCCTGGACAGGGTCTGGAATG
 GATTGGATACATTAATCCTAGCCGTGGTTATACTAATTACAATCAGAAAGTTCAAGGACAAGGCCA
 CATTGACTACAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGA
 CTCTGCAGTCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGGGCCAAG
 20 GCACCACTCTCACAGTCTCCTCACTTTCCGCGGACGATGCGAAAAAGGATGCTGCGAAGAAAGA
 TGACGCTAAGAAAGACGATGCTAAAAAGGACCTGGACATTTCAGCTGACCCAGTCTCCAGCAATC
 ATGTCTGCATCTCCAGGGGAGAAGGTCACCATGACCTGCAGAGCCAGTTCAAGTGTAAGTTACA
 TGAACCTGGTACCAGCAGAAGTCAGGCACCTCCCCAAAAGATGGATTTATGACACATCCAAAGT
 GGCTTCTGGAGTCCCTTATCGCTTCACTGOCAGTGGGTCTGGGACCTCATACTCTCTACAATCA
 25 GCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCAC
 GTTCGGTGTGGGACCAAGCTGGAGCTGAAAGATTATAAGGACGATGATGACAAATAGCTCGAG
 CGG (SEQ ID NO: 358)

METPAQLLFLLLLWLPDTTGEIVMTQSPATLSVSPGERATLSCRASQSVDDNNLVWYQQKPGQAPRLLIY
 GASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQYNNWPPWTFGQGTKVEIKLSADDAKKDA
 30 AKKDDAKKDDAKKDLQVQLQESGPGLVKPSQTLSTCTVSGGSISFNYYWSWIRHHPGKGLEWIGYI
 YYSYSTYNSPSLKSRTISVDTSKNQFSLTSSVTAADTAVYYCARGYNWNYFDYWGQGLTVTVSSLS
 ADDAKKDAKKDDAKKDDAKKDLDIKLQSGAELARPGASVKMSCKTSGYTFTRYTMHWWVKQRPG
 QGLEWIGYINPSRGYTNYNQKFKDKATLTDDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYW
 GQGTTTLTVSSLSADDAKKDAKKDDAKKDDAKKDLDIQLTQSPAISASPGKVTMTCRASSSVSYM
 35 NWYQQKSGTSPKRWIYDTSKVASGVPYRFGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAG
 TKLELKDYKDDDDK (SEQ ID NO: 359)

4. Testing of the 3 CR011 engineered antibodies by ELISA, flow cytometry and
 determination of cytotoxicity:

ELISA: The binding of the CR011 engineered antibodies to purified recombinant GPNMB (2 µg/mL) was measured using plates coated overnight at 4°C. Plates were then blocked and washed. Various dilutions of the CR011 engineered antibodies were added into the wells. Plates were incubated for 1 h and washed. HRP-conjugated anti-FLAG M2 mAb (Sigma, St. Louis, MO) was added into the wells for 1 h, washed and the reaction developed with the TMB substrate reagent as described by the manufacturer (Pharmingen, San Jose, CA).

Binding of the CR011 scFv and CR011 x anti-CD3 (L4-L2-L4 linker set) bi-scFv product to GPNMB was first confirmed using ELISA, as shown in Figure 18. Plates were coated with human GPNMB protein tagged with His and V5. Coated plates were incubated with either supernatants containing CR011 X anti-CD3 bi-scFv or purified CR011 scFv monomer. Binding of the recombinant mAbs (both monomer and dimer) was detected using anti-FLAG-HRP conjugated mAb M2 (Sigma). As can be seen in Figure 18, both anti-GPNMB antibody species described bind to the recombinant GPNMB protein, indicating that the specificity and binding activity of the engineered anti-GPNMB antibody, using the methods described in this example, was preserved.

Flow cytometry: The binding of the CR011 engineered antibodies to native proteins was analyzed by FACS. Briefly, human T cells and SK-Mel-5 cells were incubated with either the CR011 scFv or CR011 x anti-CD3 (L4-L2-L4 linker set) bi-scFv (5 µg/sample/100 µl) with subsequent staining with mouse anti-FLAG mAb (Sigma) and PE-conjugated goat anti-mouse Ig F(ab')₂. (Jackson ImmunoResearch, West Grove, PA) Ten thousand events were collected and analyzed on a FACSCalibur instrument (Becton Dickinson, Mountain View, CA).

To confirm binding of the CR011 scFv and CR011 x anti-CD3 (L4-L2-L4 linker set) bi-scFv products to native GPNMB protein expressed on the cell surface, we used SK-Mel-5 cells which naturally express GPNMB. To verify binding of the bi-scFv to human CD3 molecules, we used purified human T cells. As a positive control we used native PE conjugated anti-CD3-PE and CR011 mAb. Binding of the CR011 scFv was detected using anti-FLAG mAb M2 with subsequent staining with PE conjugated anti mouse IgG, while for detection of CR011 mAb binding we used anti-human IgG-PE. Control anti-CD3 mAb bound to T cells, and control anti-GPNMB mAb bound to SK-Mel-5 tumor cells. We found that only the CR011 x anti-CD3 (L4-L2-L4 linker set) bi-scFv stained T cells; the CR011

scFv monomer did not bind CD3 positive T cells, as expected (see Figure 19). Binding to SK-Mel-5 cells by either the CR011 scFv monomer or CR011 X anti-CD3 (L4-L2-L4 linker set) bi-scFv was present at a low level (Figure 19).

5 Cytotoxicity: The ability of CR011 x anti-CD3 (L4-L2-L4 linker set) bi-scFv to redirect human T lymphocytes to kill relevant human tumor cells was measured by flow cytometry. Tumor cells were labeled with PKH2 green fluorescent linker kit (Sigma) and washed. Purified T cells were cultured O/N with PKH2-labeled tumor cells in the presence or absence of purified bi-scFv. Death of GPNMB positive tumor cells was measured by propidium iodine (PI) incorporation.

10 To evaluate the ability of the CR011 x anti-CD3 (L4-L2-L4 linker set) bi-scFv product to increase T cell mediated killing of GPNMB positive cells, we performed a cytotoxicity test. Purified T cells were cultured O/N with PKH2-labeled SK-Mel-5 (GPNMB positive) tumor cells in the presence of various doses of purified CR011 scFv and CR011 x anti-CD3 (L4-L2-L4 linker set) bi-scFv products.

15 Conclusion:

 The CR011 x anti-CD3 (L4-L2-L4 linker set) bi-scFv significantly increased killing of SK-Mel-5 tumor cells by T lymphocytes (Figure 20). In contrast, the addition of mono-specific anti-GPNMB scFv did not increase killing of SK-Mel-5 tumors. In addition, no cytotoxicity was observed when the tumor cells were cultured with the CR011 x anti-CD3
20 (L4-L2-L4 linker set) bi-scFv without T lymphocytes (Figure 20). These data indicate that the CR011 x anti-CD3 (L4-L2-L4 linker set) bi-scFv provided sufficient bridging between T cells and SK-Mel-5 cells to induce cell death and that both components of this engineered CR011 bi-specific antibody were biologically active. Therefore the CR011 x anti-CD3 (L4-L2-L4 linker set) bi-scFv engineered antibody of the present invention may be used as a
25 therapeutic to treat diseases, such as melanoma and other cancers where there are upregulated levels of GPNMB and T cells present.

 Other methods of cytotoxicity analysis, including fluorescence and chromium release assays can be used to demonstrate the usefulness of the CR011 x anti-CD3 (L4-L2-L4 linker set) bi-scFv in treating tumors. Other linkers may also be used to link the two
30 scFv monomer components together, as in the CR011 x anti-CD3 (linker set L4-L4-L4) molecule described *supra*.

Example 29: Optimized Production Process of CR011-vcMMAE

CR011AE is an antibody-drug conjugate composed of the anti-GPNMB (CG56972) fully human antibody CR011 conjugated with the toxin Auristatin E through a protease-cleavable linker. The toxin-to-antibody ratio is approx. 4.0 but may vary between 3.5 and 4.2. While the CR011 antibody is IgG2, it is therefore possible to append up to 12 toxin molecules per antibody molecule using the free thiols as a reactive site.

The structure of Maleimidocaproyl-Valine-Citrullin-Monomethyl-Auristatin E (vcMMAE) is shown in Figure 21.

Conjugation: A process of generating the drug-substance consisting of CR011 mAb with VCMAE attached. An overview of the conjugation process is summarized in Figure 22.

Briefly, the conjugation process for CR011 fully human antibody consists of the following 4 steps. 1) Buffer exchange and sucrose removal by diafiltration, 2) Disulfides reduction, 3) Conjugation to vcMMAE and finally, 4) Purification of conjugated CR011-vcMMAE by diafiltration. There are several assays throughout the process, *i.e.* in-process assays, which include Ellman's assay and determination of protein concentration. At the end of the process, the drug substance, *i.e.* the conjugate, is analyzed for drug-to-antibody ratio, free drug content and protein concentration.

Diafiltration of the bulk antibody: The bulk antibody originally formulated in phosphate pH 7 -10% sucrose was buffer exchanged into the conjugation buffer (borate pH 9.0 – NaCl) by diafiltration over 10 diavolumes. At the end of diafiltration, CR011 was diluted to ~5.5 mg/mL and filtered through a set of two filters consisting of 1.2 and 0.22 μ m. The buffer exchange is required because sucrose interferes with reduction. In addition, high pH improves CR011 solubility.

CR011 reduction - General considerations: CR011 is produced as an IgG2 isotype product and contains 6 disulfide bridges in the hinge region. These disulfides can be reduced under mild conditions to give rise to 12 cysteine residues. Therefore, it is possible to maximally attach 12 vcMMAE drug molecules per antibody. For the process, however, the bulk antibody is only partially reduced because the aim is to generate conjugates with an average of 4 vcMMAE molecules. The reason for this is two-fold. First, it broadens the therapeutic window by decreasing potential systemic toxicity associated with MMAE.

Second, it is difficult and sometimes impossible to produce fully-loaded conjugates with low aggregation because of greatly reduced solubility imparted by the hydrophobic drug.

Process: Tris-(carboxyethyl)-phosphine or TCEP was added at the 4:1 molar ratio (TCEP:mAb) to CR011 at a concentration of ~5.5 mg/mL in the jacketed reactor equipped with an agitator set to 90 RPM. The reaction was allowed to proceed for 3 hours at 37°C in the presence of 1 mM EDTA. At the end, Ellman's assay was used to determine the amount of free thiols. Typically, it was 4.2 thiols per antibody. The reactor was then chilled to 4°C.

CR011 conjugation - General considerations: TCEP was not fully consumed during the reduction. The left over TCEP was capable of reacting with vcMMAE. However, this spurious side reaction was slower compared to the conjugation reaction and can be mitigated by adding an excess of vcMMAE. The advantage of TCEP compared to DTT is that it does not require removal of the left-over reducing agent.

Process: vcMMAE was dissolved in DMSO and added at 20% molar excess to the reduced CR011 mAb. The reaction was allowed to proceed for 1 hour. The final concentration of DMSO is 4% (v/v). DMSO played a dual purpose in the process. It is required for solubilizing the drug and also it helps to solubilize the conjugate. At the end of conjugation, N-acetylcysteine was added to quench any unreacted drug.

CR011-vcMMAE purification: The temperature in the reactor was brought to room temperature. A 40% sucrose stock solution was used to adjust the final sucrose concentration to 10% (w/v) followed by a pH adjustment using 300 mM histidine HCl pH 5.0 buffer to a final pH of 6.0. The conjugate was then purified by diafiltration into 20 mM histidine pH 6.0-10% sucrose (w/v) buffer and using 10 diavolumes. At the end of diafiltration, the conjugate was concentrated to ~7 mg/mL and filtered through a set of three filters consisting of 1.2, 0.45 and finally, 0.22 µm.

CR011-vcMMAE formulation: The conjugate was formulated by adding Tween-20 to a final concentration of 0.02% and by diluting to 6 mg/mL ($\pm 10\%$) using formulation buffer (20 mM histidine pH 6.0, 10% sucrose, 0.02% Tween-20). The conjugate was then stored at 4°C until pooling if more than one lot is being manufactured (a.k.a. staging time). After pooling, the final concentration was adjusted to 5.0 mg/mL ($\pm 5\%$) and the drug substance was stored frozen.

1. Pre-conjugation UF/DF: Removal of Sucrose

Experiments were performed to monitor the rate of removal of sucrose during UF/DF by Ellman's assay; and estimate the diavolumes needed to achieve the highest SH-per-Ab ratio.

- 5 It was found that it is desirable to conduct at least 6 diavolumes in order to remove sucrose to a level that does not impede CR011 reduction. To ensure robustness, at least 10 diavolumes should be utilized during the process.

2. The Effect of Percent DMSO on Aggregation in the Conjugation Reaction

Experiments were performed to determine the effect of DMSO in the conjugation reaction on: (1) aggregation; and (2) drug:Ab molar ratio (*i.e.* completeness of conjugation).

- 10 It was found that the percent aggregate in reaction with 12% DMSO was lower than in 15% DMSO, 4.4 and 3.0%, respectively. Formulation pH 9.0 buffer vs. pH 7.0 buffer did not have any effect on aggregation or yield, provided 10% sucrose was included in formulation. The percent aggregate in the 10%, 8%, 6%, and 4% (v/v) DMSO reactions were 2.7, 1.7, 1.0 and 0.5%, respectively. This suggests that CR011 and CR011AE were very susceptible to aggregation when a higher percentage of DMSO is present.

All four conjugation reactions resulted in a final molar ratio of 4.0 drugs/Ab, suggesting that all four reactions went to completion. Safety margins for DMSO percentage in the conjugation reaction are 4 – 6%. This predicted to yield an aggregation level of 1% or less.

5. Investigation of Side Reaction During Conjugation of CR011 to vcMMAE

- Experiments were performed to: (1) investigate the extent and the kinetics of the side reaction in which maleimide-drug is converted into an unreactive side product, which results in an incomplete conjugation and low drug-loading; (2) determine factors that influence the side reaction; and (3) determine whether the old vcMMAE lot (SGD1006-0-04) differed in reactivity compared to the new lot (SGD1006-0-06).

Reactions (100 μ l) containing vcMMAE at 30 μ M final concentration, were incubated in borate pH 9.0 buffer either in the absence or in the presence of 2-fold molar excess of TCEP (with respect to vcMMAE). The reactions were quenched at 0, 2, 7 or 15

min with excess NAcCys. The control consisted of vcMMAE in phosphate pH 7.0 buffer quenched at the 15 min time point. The chromatograms are shown on Figure 23.

In pH 7 phosphate buffer 15 min and in pH 9.0 borate buffer 0 min after addition of the drug a single Cys-quenched product with (rt = 9.0 min) is formed (Compare A and B).

- 5 In borate buffer pH 9.0 an unreactive side product is formed (rt=9.2 min) in a time-dependent fashion (B, C, D and E). In borate buffer and in the presence of TCEP (such as CR011 conjugation conditions), formation of the unreactive product is catalyzed resulting in >90% conversion of maleimide into succinimide after only 2 min of incubation (F through I). Both the old vcMMAE lot (SGD-1006-0-06) and the new lot (SGD-1006-0-04)
- 10 exhibited similar reactivity towards high pH and TCEP, as well as similar kinetics.

Figure 24 shows the LC-MS identification of the unreactive product as succinimidyl-VCMAE (rt = 9.2 min, m/z = 1318). Figure 25 shows the relative kinetics of formation of the succinimide in the presence or absence of TCEP.

Conclusions

- 15 The side product is a result of conjugation performed at pH 9 instead of pH 7.4 (PBS). Formation of the side product is greatly enhanced in the presence of TCEP. The major stable side product has been identified by LC-MS as succinimidyl-vcMMAE. Minor and less stable side-products remain to be identified. Both vcMMAE lots behaved similarly.

- 20 6. Overcoming the Side Reaction During Conjugation of CR011 to vcMMAE

Experiments were performed to investigate whether the side reaction can be overcome by providing a larger excess of the drug for conjugation.

- Several ways to suppress the side reaction were proposed: (1) Conducting conjugation at lower pH, *e.g.* 8.5 instead of 9.0 (high risk due to reduced solubility of CR011); (2) Removal of the excess of TCEP by UF/DF (not practical); and (3) Elevation of the excess of VCMAE added upfront (practical).
- 25

- 100 mg of CR011 that was previously buffer exchanged into 50 mM borate-50 mM NaCl, was reduced with TCEP to generate 4.35 free thiols per Ab. The reaction was divided into two halves. For the first 50 mg half, a 10% excess of VCMAE was added based on the 1 drug per thiol ratio. For the second half, a 20% excess was used. The conjugates were
- 30

purified by UF/DF into 10 mM Histidine pH 6/ 10% sucrose solution. The results are summarized in Table 60.

Table 60. Preparation of CR011-VCMAE conjugates using various excess of vcMMAE based on the 1 drug per thiol ratio. Drug-to-Ab ratios were determined by RP HPLC.

	VCMAE Excess, %	
	10	20
SH per Ab	4.35	4.35
Drug-to-Ab ratio (in reaction)	3.9	4.1
Drug-to-Ab ratio (in final product)	3.7	4.0

Conclusions

Using 10% versus 20% excess of vcMMAE was compared in a 100 mg conjugation. The higher excess of vcMMAE afforded a drug-to-Ab ratio closer to the expected value, and therefore, has been determined to be optimal.

Equivalents

The foregoing description and Examples detail certain preferred embodiments of the antibodies and describes the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the methods of making and using the antibodies described herein may be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof. The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the embodiments described herein.

CLAIMS

1. An isolated human antibody that specifically binds to GPNMB.
2. The antibody of claim 1 in which the antibody is a monoclonal antibody.
- 5 3. The antibody of claim 1 in which the antibody specifically binds GPNMB with an affinity constant greater than 10^7M^{-1} .
4. The antibody of claim 1 comprising a region selected from the group comprising: VH1-2, VH2-5, VH3-11, VH3-21, VH3-30, VH3-33, VH4-31, VH4-59 and VH5-51.
5. The antibody of claim 4 further comprising a region selected from the group
10 comprising: A2, A3, A20, A27, A30, L2 and O1.
6. The antibody of claim 1 comprising a region derived from a region selected from the group comprising: VH1-2, VH2-5, VH3-11, VH3-21, VH3-30, VH3-33, VH4-31, VH4-59 and VH5-51.
7. The antibody of claim 6 further comprising a region derived from a region selected
15 from the group comprising: A2, A3, A20, A27, A30, L2 and O1.
8. The antibody of claim 1 comprising an amino acid sequence selected from the group comprising: SEQ ID NO: 2, 20, 38, 56, 74, 92, 110, 128, 146, 164, 182, 200, 218, 236, 253, 256, 260, 265, 270, 274, 277, 281 and 285.
9. The antibody of claim 8 further comprising an amino acid sequence selected from
20 the group comprising: SEQ ID NO: 11, 29, 47, 65, 83, 101, 119, 137, 155, 173, 191, 209, 227 and 245.
10. The antibody of claim 1 comprising a CDR comprising an amino acid sequence selected from the group comprising: SEQ ID NO: 4, 6, 8, 13, 15, 17, 22, 24, 26, 31, 33, 35, 40, 42, 44, 49, 51, 53, 58, 60, 62, 67, 69, 71, 76, 78, 80, 85, 87, 89, 94, 96,
25 98, 103, 105, 107, 112, 114, 116, 121, 123, 125, 130, 132, 134, 139, 141, 143, 148, 150, 152, 157, 159, 161, 166, 168, 170, 175, 177, 179, 184, 186, 188, 193, 195, 197, 202, 204, 206, 211, 213, 215, 220, 222, 224, 229, 231, 233, 238, 240, 242, 247, 249, 251, 254, 257, 261, 266, 271, 278, 282, 286, 255, 258, 262, 267, 272, 275, 279, 283, 287, 259, 263, 264, 268, 269, 273, 276, 280, 284 and 288.
11. The antibody of claim 1 wherein the antibody is selected from the group comprising: Mab1.10.2, Mab1.15.1, Mab1.2.2, Mab1.7.1, Mab2.10.2, Mab2.15.1, Mab2.16.1, Mab2.17.1, Mab2.21.2, Mab2.22.1, Mab2.24.1, Mab2.3.1, Mab2.7.1 and Mab2.8.1.
12. The antibody of any of the claims 1-11, wherein said antibody is an IgG1 antibody.

13. An immunoconjugate comprising the antibody of claim 1 and a cytotoxic agent.
14. The immunoconjugate of claim 13, wherein said antibody is Mab1.15.1.
15. The immunoconjugate of claim 13, wherein the cytotoxic agent is auristatin E (dolastatin-10) or a derivative thereof.
- 5 16. A pharmaceutical composition comprising the immunoconjugate of claim 13.
17. A pharmaceutical composition comprising the antibody of claim 12, and an immunomodulator.
18. An antibody comprising human framework regions and means for specific binding to GPNMB.
- 10 19. The antibody of claim 18, wherein the means comprises a CDR comprising or derived from SEQ ID NO: 4, 6, 8, 13, 15, 17, 22, 24, 26, 31, 33, 35, 40, 42, 44, 49, 51, 53, 58, 60, 62, 67, 69, 71, 76, 78, 80, 85, 87, 89, 94, 96, 98, 103, 105, 107, 112, 114, 116, 121, 123, 125, 130, 132, 134, 139, 141, 143, 148, 150, 152, 157, 159, 161, 166, 168, 170, 175, 177, 179, 184, 186, 188, 193, 195, 197, 202, 204, 206, 211, 213, 15 215, 220, 222, 224, 229, 231, 233, 238, 240, 242, 247, 249, 251, 254, 257, 261, 266, 271, 278, 282, 286, 255, 258, 262, 267, 272, 275, 279, 283, 287, 259, 263, 264, 268, 269, 273, 276, 280, 284 and 288.
20. An isolated nucleic acid encoding the antibody of claim 1.
21. An expression vector comprising the nucleic acid of claim 20.
- 20 22. A host cell comprising the vector of claim 21.
23. The host cell of claim 22, wherein the host cell is an *E. coli* bacterium, a Chinese hamster ovary cell, a HeLa cell, or a NSO cell.
24. The nucleic acid of claim 23, wherein the nucleic acid encodes the amino acid sequence selected from the group comprising: SEQ ID NO: 2, 20, 38, 56, 74, 92, 25 110, 128, 146, 164, 182, 200, 218, 236, 253, 256, 260, 265, 270, 274, 277, 281 and 285.
25. The nucleic acid of claim 24, wherein the nucleic acid comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, 19, 37, 55, 73, 91, 109, 127, 145, 163, 181, 199, 217, 235, 10, 28, 46, 64, 82, 100, 118, 136, 154, 172, 30 190, 208, 226 and 244.
26. A method of making a human monoclonal antibody that specifically binds with GPNMB, the method comprising the steps of: (a) providing a starting repertoire of nucleic acids encoding a variable domain that either includes a CDR3 to be replaced

- or lacks a CDR3 encoding region; (b) combining the repertoire with a donor nucleic acid encoding an amino acid sequence substantially as set out in the sequence selected from the group comprising: SEQ ID NO: 8, 26, 44, 62, 80, 98, 116, 134, 152, 170, 188, 206, 224, 242, 259, 263, 264, 268, 269, 273, 276, 280, 284 and 288 such that the donor nucleic acid is inserted into the CDR3 region in the repertoire, so as to provide a product repertoire of nucleic acids encoding a variable domain; (c) expressing the nucleic acids of the product repertoire; (d) selecting an antigen-binding fragment specific for GPNMB; and (e) recovering the specific antigen-binding fragment or nucleic acid encoding the binding fragment.
- 5
27. An antibody produced by the method of claim 26.
- 10
28. An antibody, or binding fragment thereof, that binds to GPNMB, wherein said antibody, or binding fragment thereof, neutralizes a GPNMB-induced activity, and wherein said antibody, or binding fragment thereof, cross-reacts with a fully human anti-GPNMB antibody selected from the group consisting of Mab1.2.1, Mab1.10.1, and Mab2.22.1 or an antibody in the same antigen-binding bin as fully human anti-GPNMB antibody Mab1.2.1, Mab1.10.1, or Mab2.22.1.
- 15
29. An antibody, or binding fragment thereof, that binds to GPNMB, wherein said antibody, or binding fragment thereof, neutralizes a GPNMB-induced activity, and wherein said antibody, or binding fragment thereof, cross-reacts with a fully human anti-GPNMB antibody selected from the group consisting of Mab2.3.1 and Mab1.15.1 or an antibody in the same antigen-binding bin as fully human anti-GPNMB antibody Mab2.3.1 or Mab1.15.1.
- 20
30. An antibody, or binding fragment thereof, that binds to GPNMB, wherein said antibody, or binding fragment thereof, neutralizes a GPNMB-induced activity, and wherein said antibody, or binding fragment thereof, cross-reacts with fully human anti-GPNMB antibody Mab2.10.1 or an antibody in the same antigen-binding bin as fully human anti-GPNMB antibody Mab2.10.1.
- 25
31. A single chain Fv antibody comprising a V_L domain of a monoclonal human anti-GPNMB antibody linked to a V_H domain of said anti-GPNMB antibody.
- 30
32. The single chain Fv antibody of claim 31 further comprising a V_H domain of an anti-CD3 antibody linked to a V_L domain of said anti-CD3 antibody.
33. An immunoconjugate comprising a single chain Fv antibody of claim 31 and a cytotoxic agent.

34. The single chain Fv antibody of claim 31 or 32 further comprising a signal peptide or a flag tag.
35. The single chain Fv antibody of claim 31 comprising the amino acid sequence of SEQ ID NO: 355.
- 5 36. The single chain Fv antibody of claim 32 comprising the amino acid sequence of SEQ ID NO: 357 or SEQ ID NO: 359.
37. A method of treating or preventing a disease associated with overexpression of GPNMB comprising administering to a subject in need thereof an effective amount of a composition comprising
- 10 (a) the antibody of claim 12;
(b) the immunoconjugate of claim 13 or 33; or
(c) the single chain Fv antibody of claim 32.
38. The method of claim 37, wherein said disease is melanoma or a neoplasm of CNS system.
- 15 39. The method of claim 38, wherein said neoplasm of CNS system is astrocytoma, glioblastoma, medulloblastoma, or neoplastic meningitis.
40. The method of claim 37, wherein said subject is human.
41. The method of claim 37, wherein said method comprises administering to said subject the immunoconjugate of claim 12, and wherein said effective amount is a
- 20 unit dose between 0.1 mg/kg to 10 mg/kg, with 2 to 4 administrations.
42. The method of claim 41, wherein said effective amount is a unit dose between 0.1 mg/kg to 2 mg/kg.
43. The method of claim 41, wherein said effective amount is a unit dose about 1 mg/kg.
44. A method of detecting GPNMB-overexpressed cells in a biology sample comprising
- 25 contacting said sample with a composition comprising the antibody of any of claims 1-11.
45. The method of claim 44, wherein said method is immunohistochemistry.

Figure 1

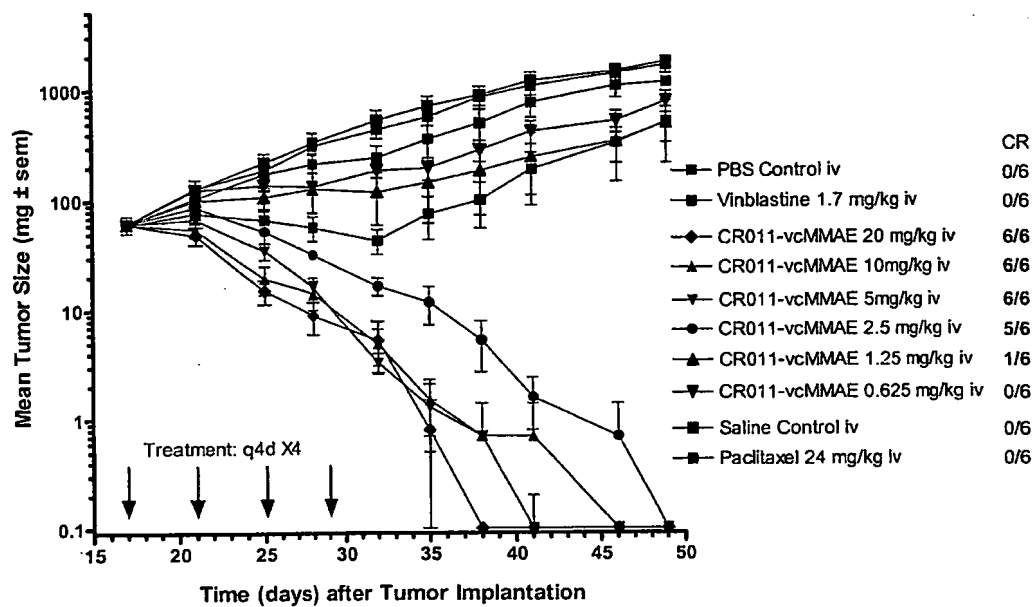
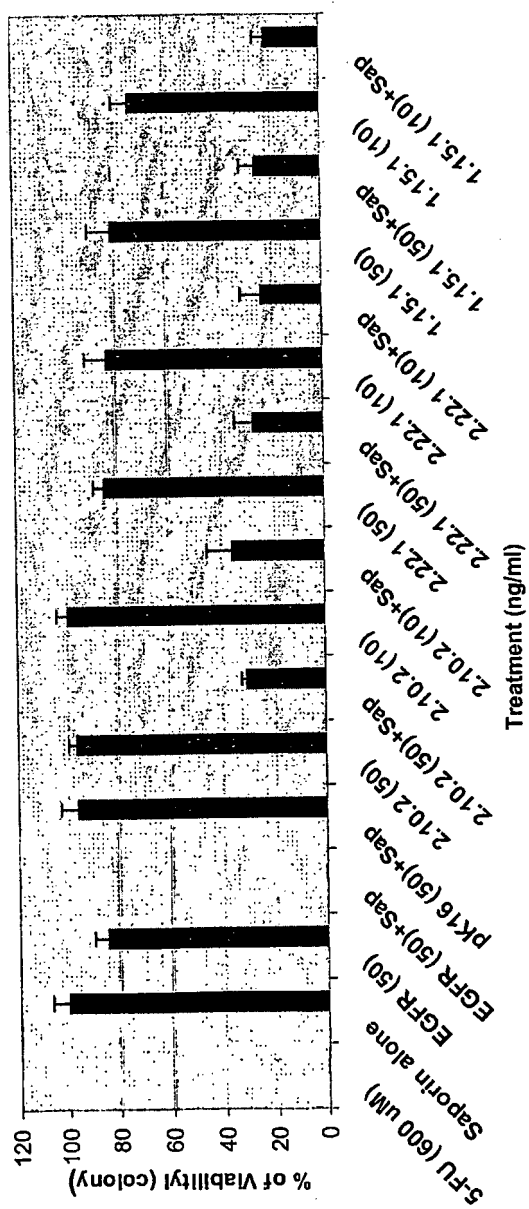


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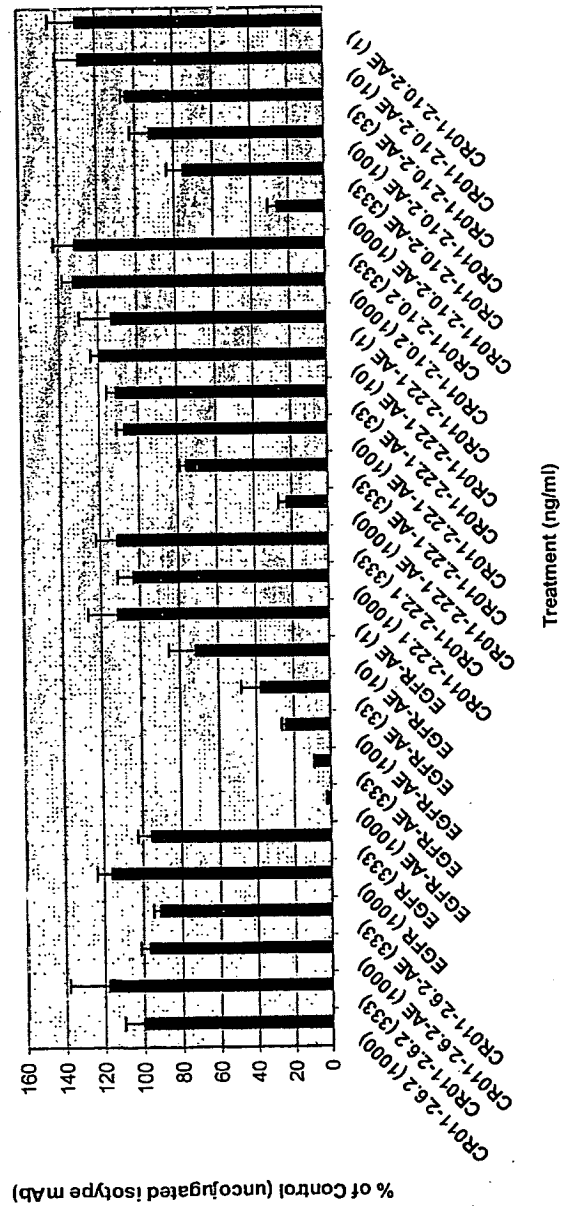


Figure 3

Figure 4

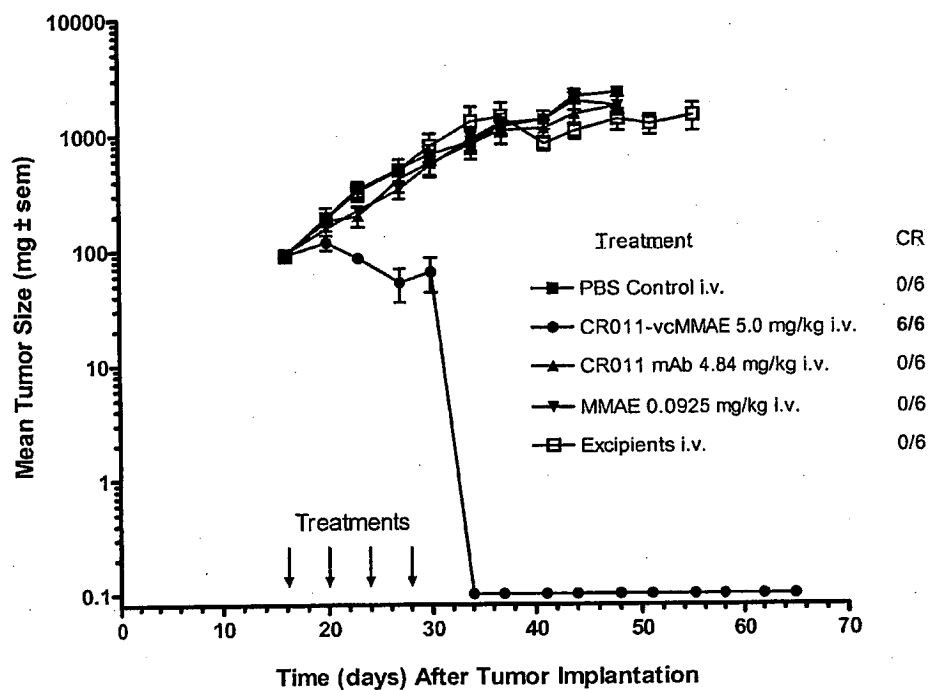


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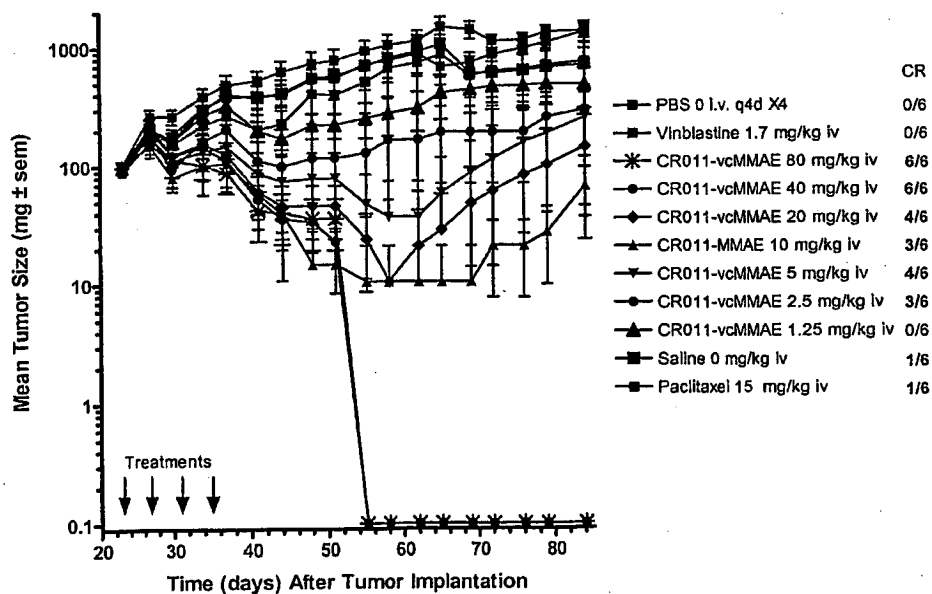


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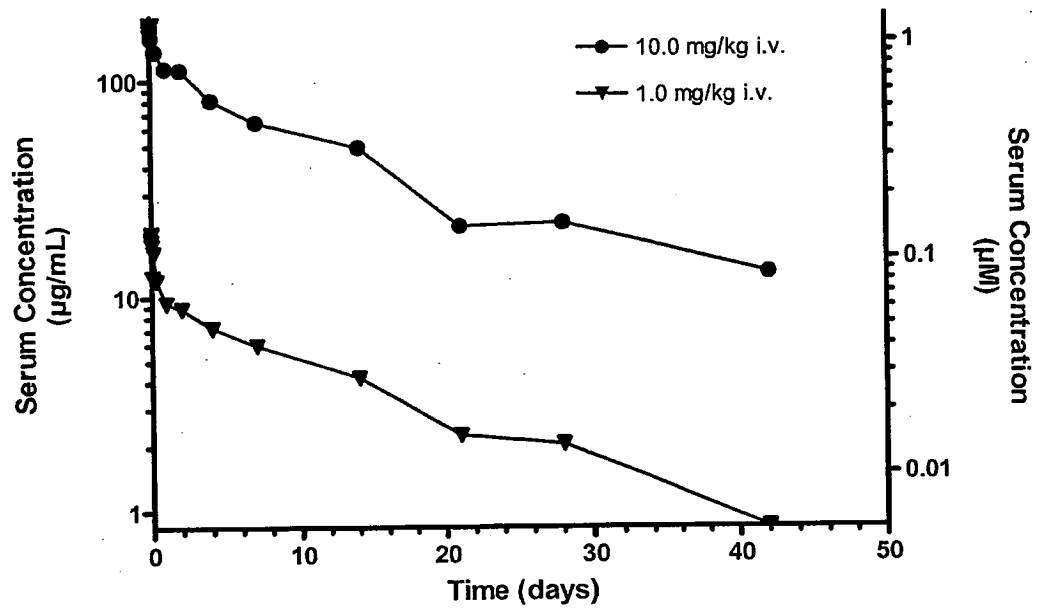


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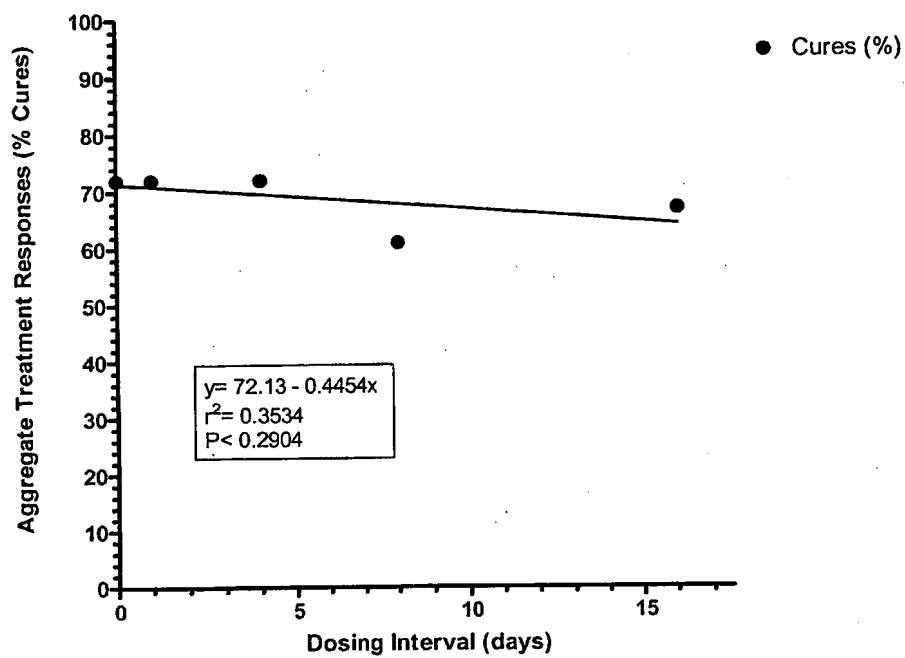


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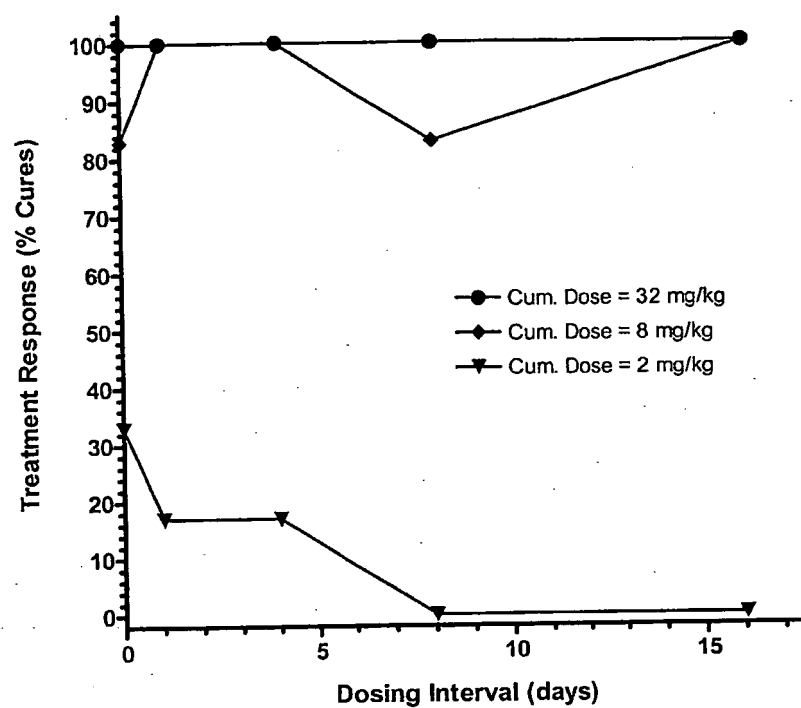


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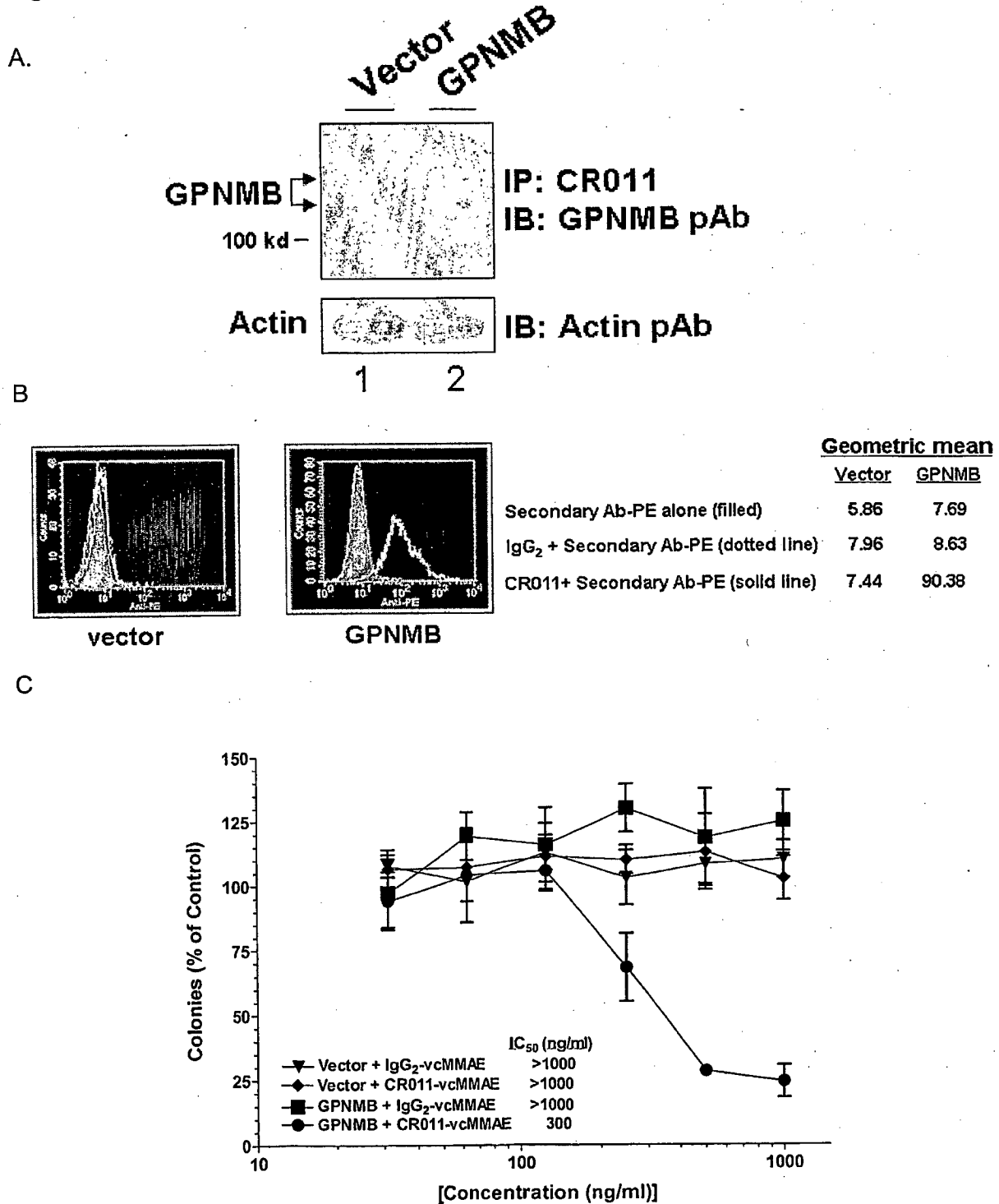
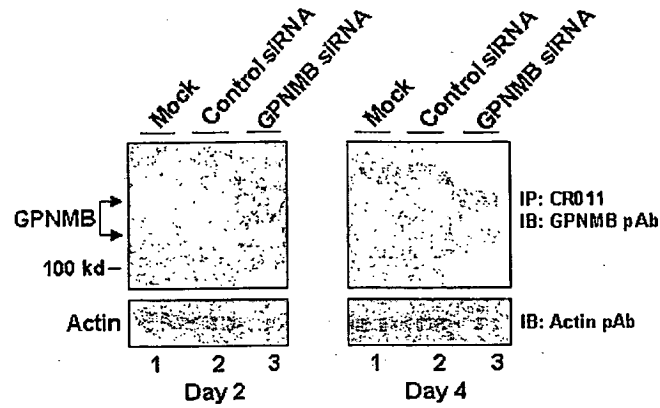
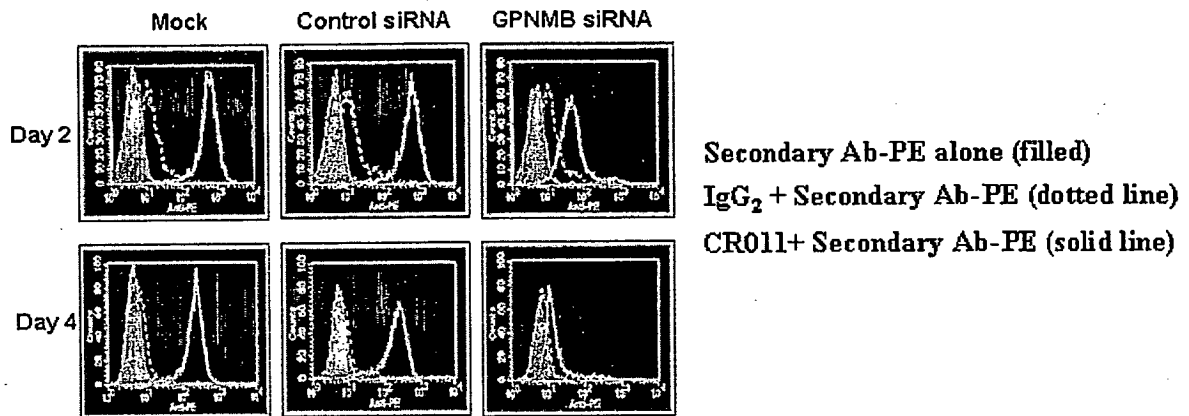


Figure 10

A



B



C

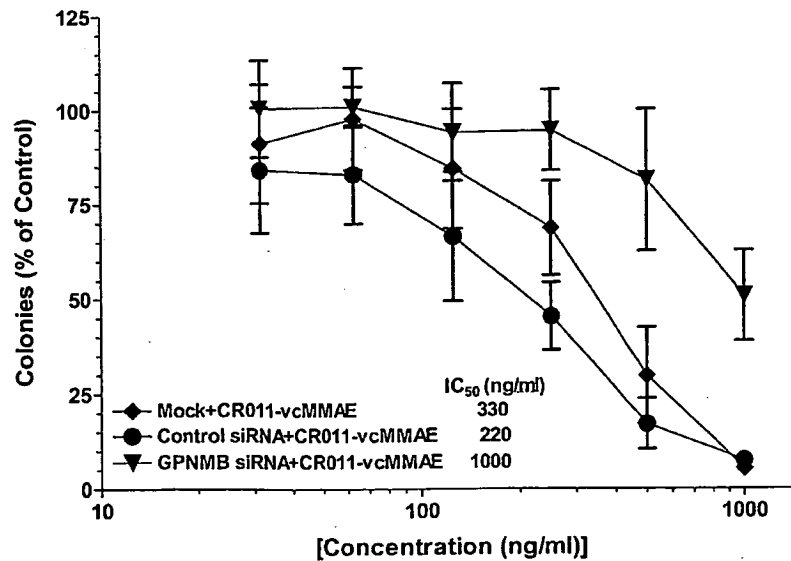
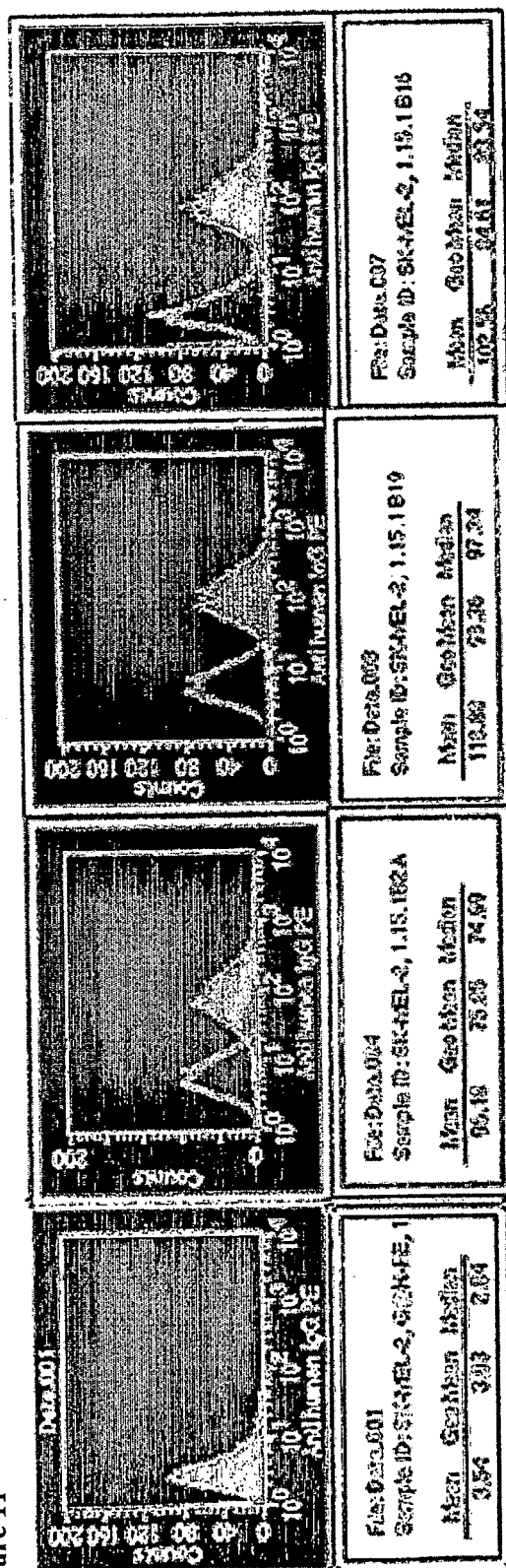


Figure 11



Control

B2: G2, Hyb

B19: G2, Recomb

B16: G1, Recomb

25

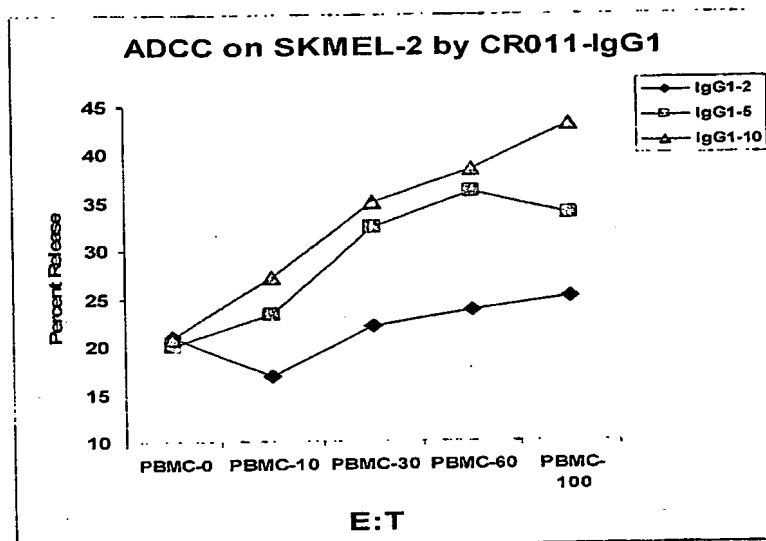
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28

GMR:

Figure 12

A.



B.

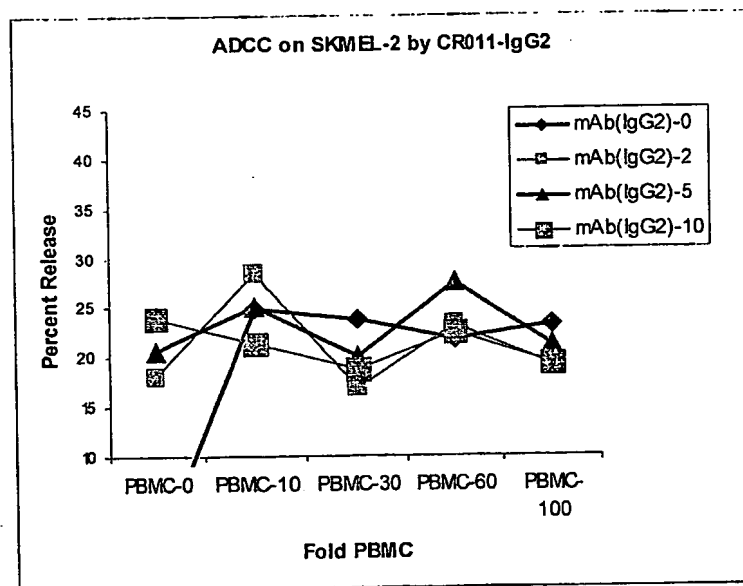


Figure 13
A.

9463_XE-03CNS	35.11	13.9%
9410_SNB-73 CNS/glioma	26.95	17.9%
9411_SF-295 CNS/glioblastoma	33.22	1%
9412_T920 Glioblastoma	30.56	1.9%
9470_SK-N-SH Neuroblastoma (metastatic)	31.78	1%
9413_SF-295 CNS/glioblastoma	39.25	17%
glioblastoma U87-MG	22.98	18.2%
glioblastoma U-118-MG	23.35	9.9%
astrocytoma SV7783	29.63	2%
neuroblast met SK-N-AS	27.83	6%
astrocytoma SF-539	22.1	33.4%
astrocytoma SNB-75	25.33	3.6%
glioma SNB-19	26.48	1.6%
glioma U251	25.44	3.3%
glioma SF-295	24.01	8.9%

B.

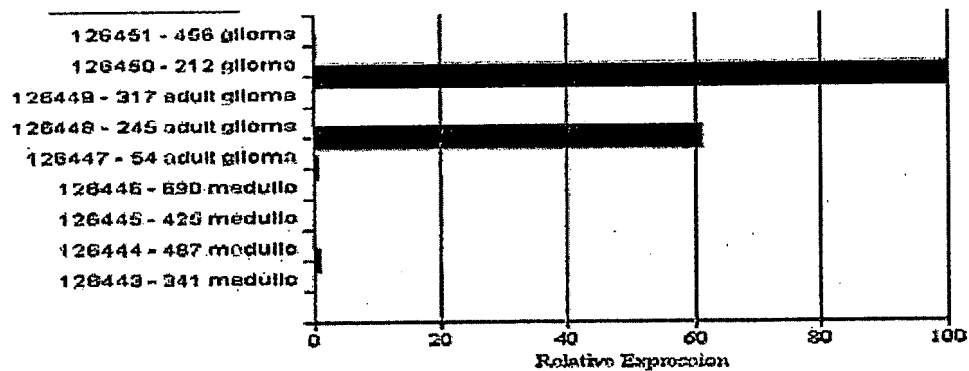


Figure 13

C

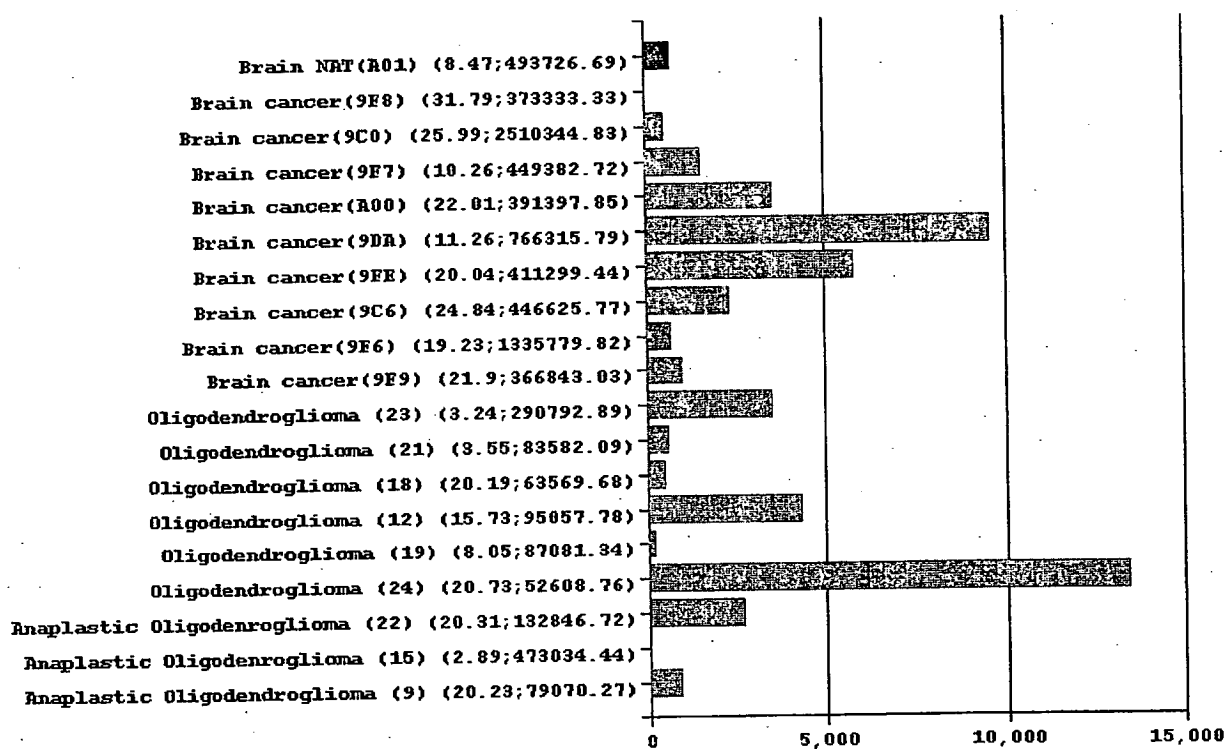
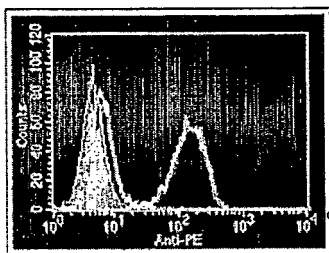
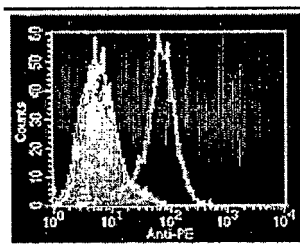


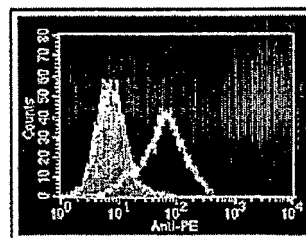
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SK-MEL-2

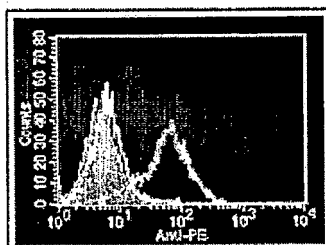
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Pink=1.15.1
21.4-fold shift

XF-498

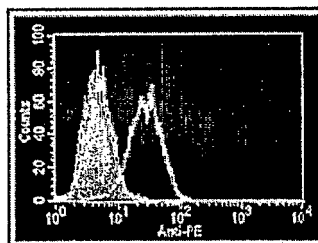
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U-118-MG

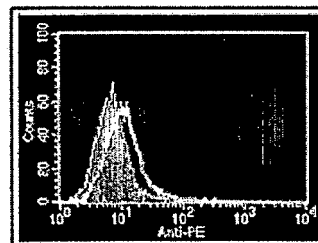
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SNB-78

Purple=anti-Human PE
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8.6-fold shift

SF-539

Purple=anti-Human PE
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SF-268

Purple=IgG2
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Figure 15

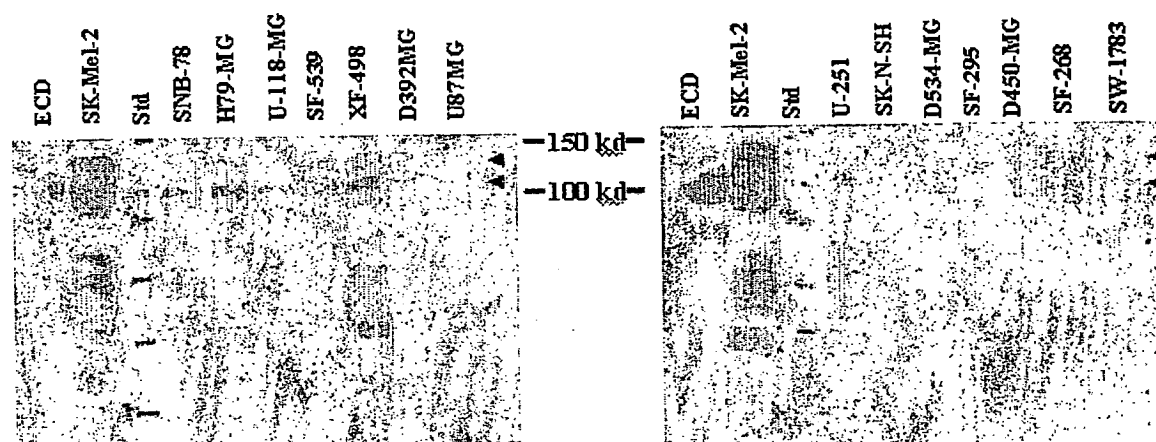


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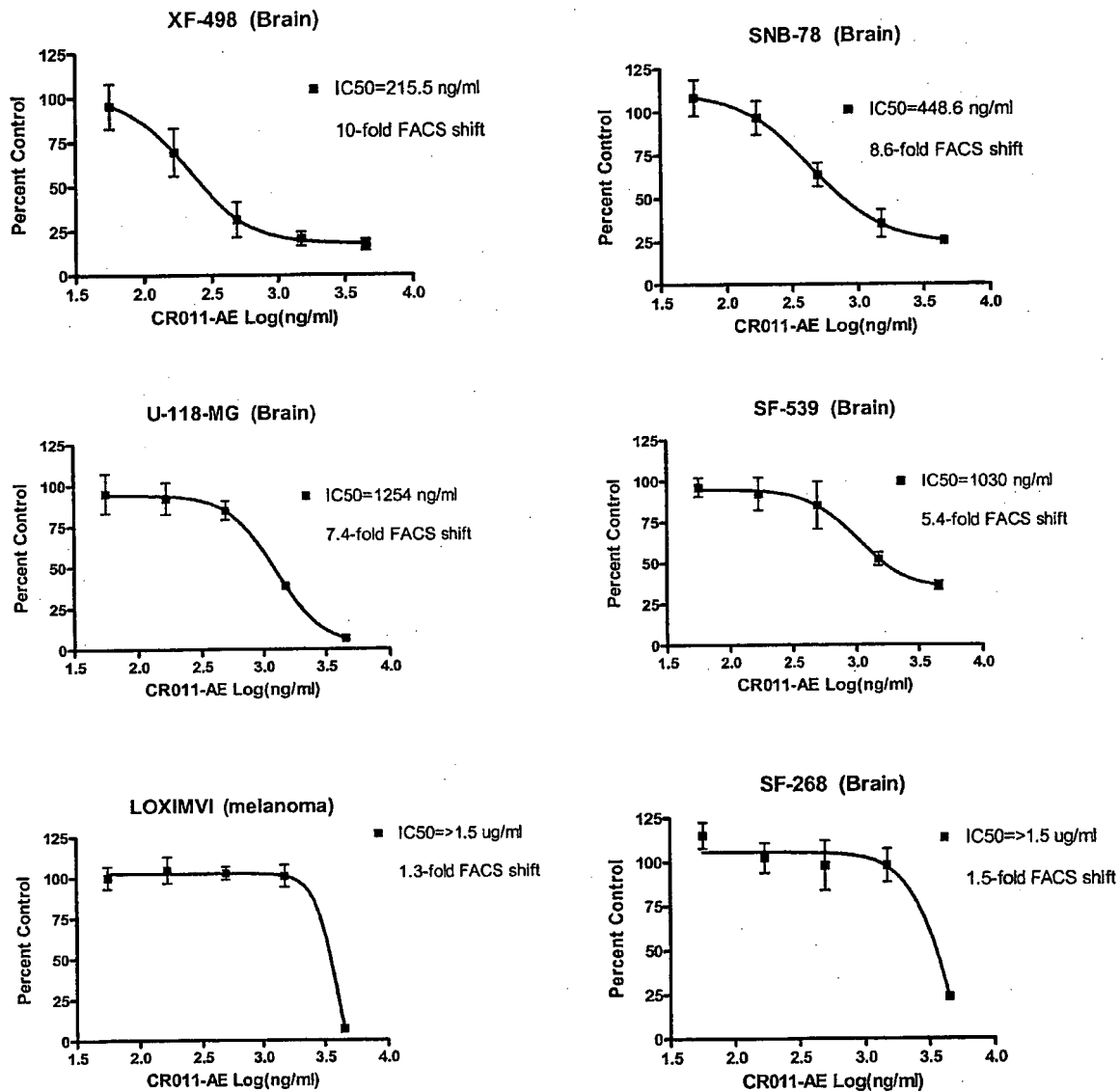


Figure 17

Development of CR011 Engineered Antibodies

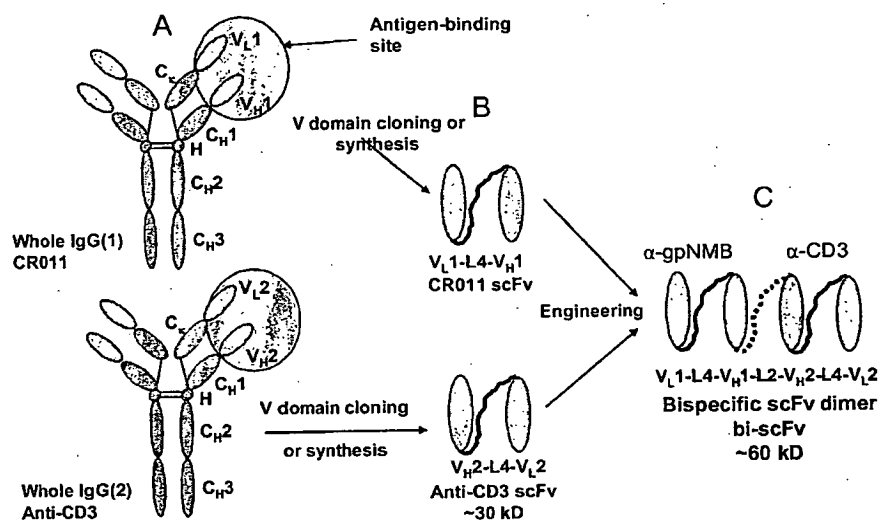
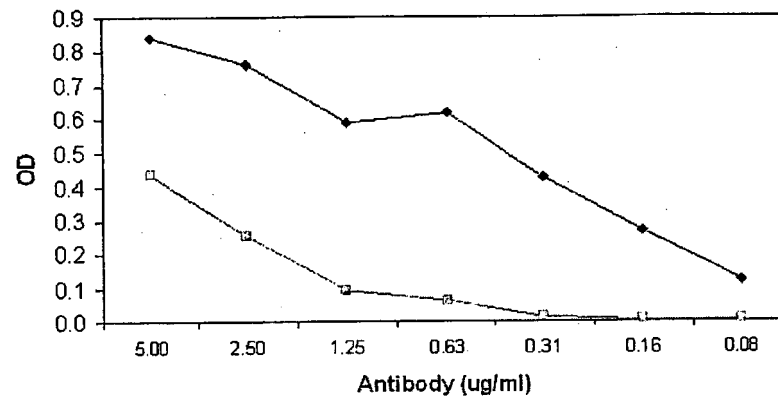


Figure 18

A



B

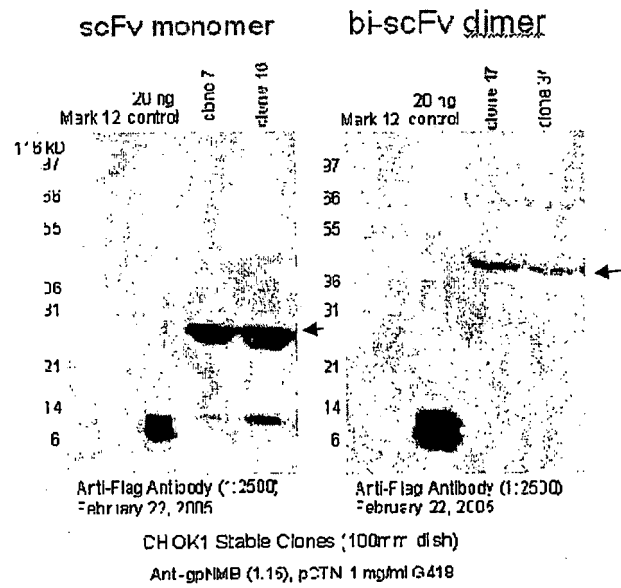


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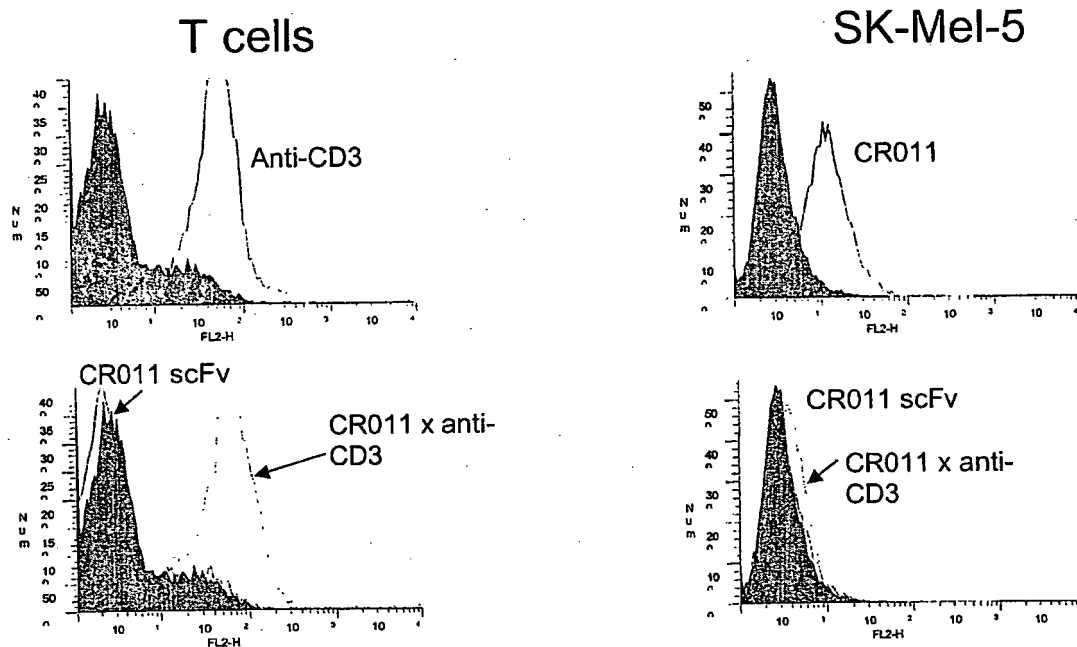


Figure 20

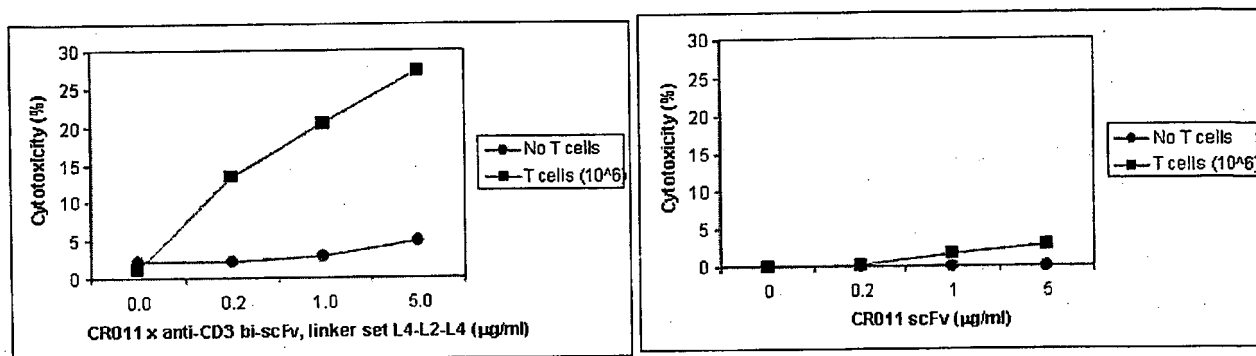


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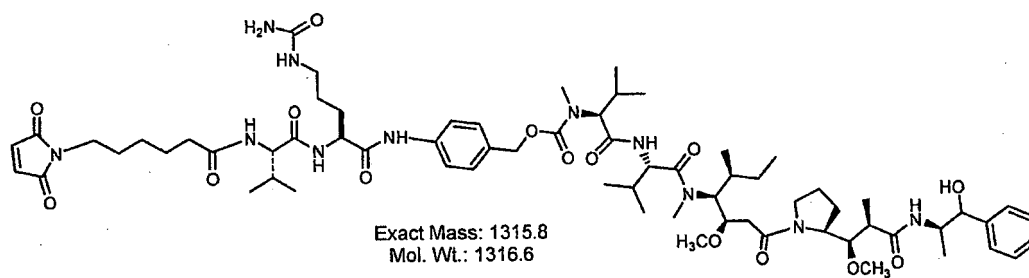


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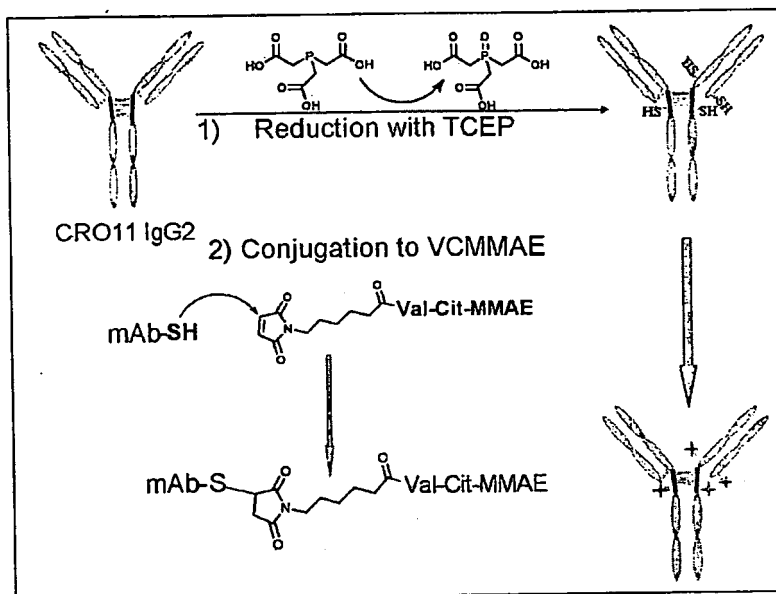


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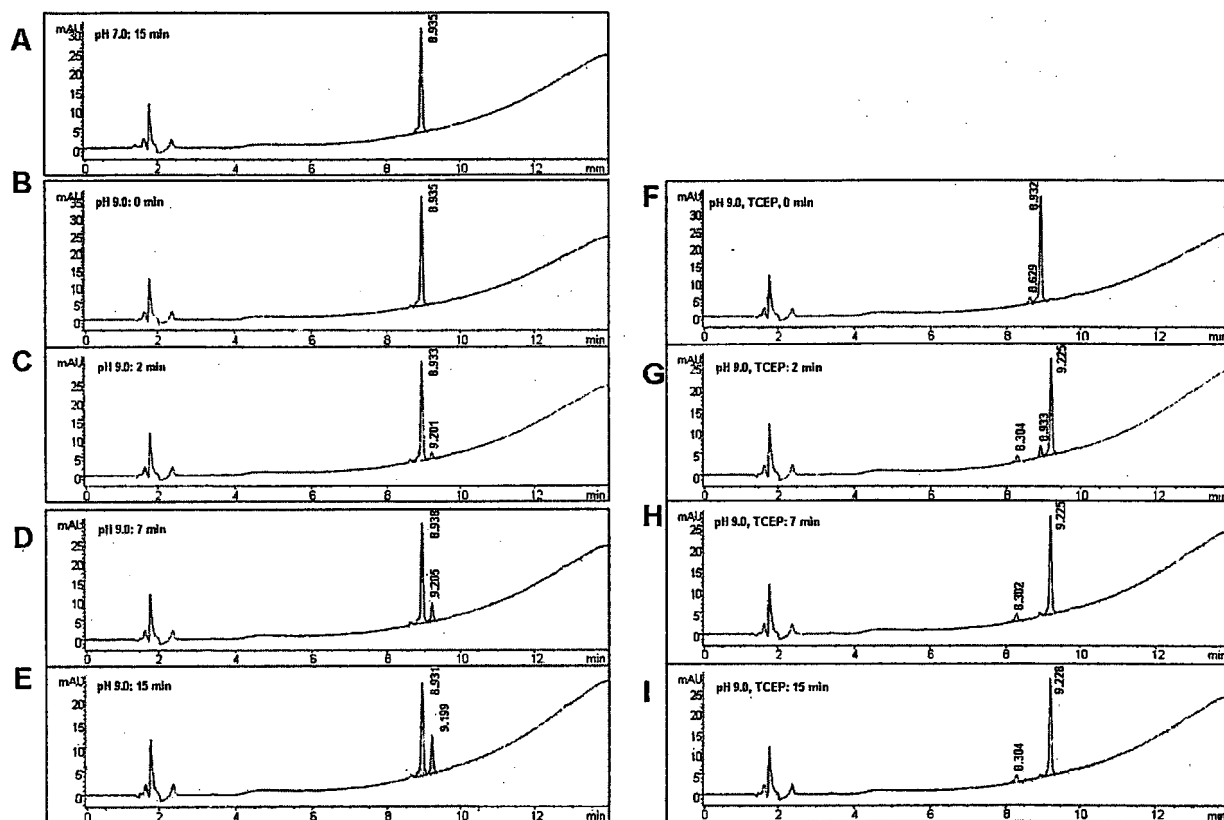


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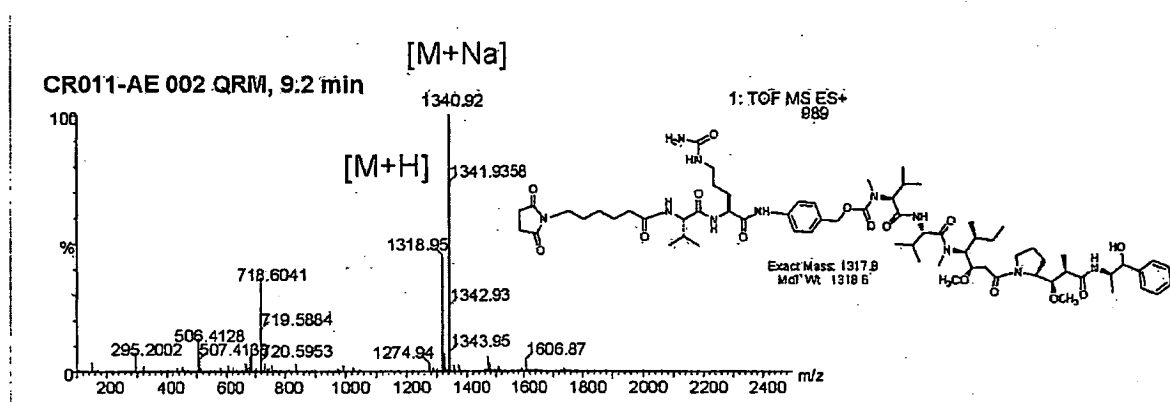
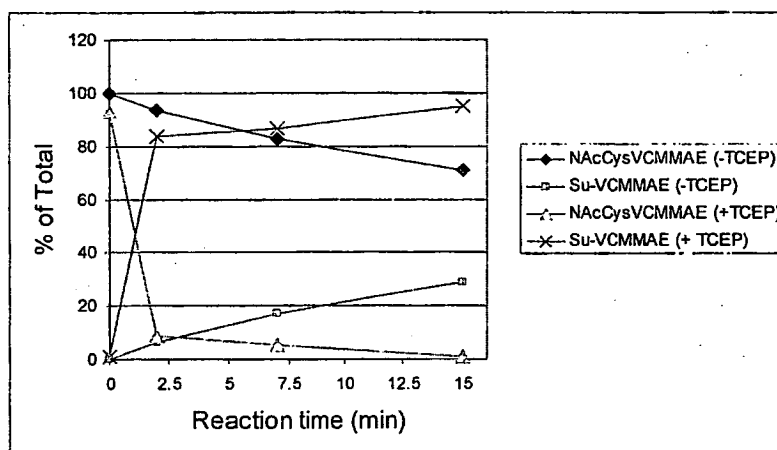


Figure 25



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Ile Thr Leu Lys Glu Ser Gly Pro Thr Leu Val Lys Pro Thr Gln Thr
 1 5 10 15

Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Ala Gly Gly
 20 25 30

Val Gly Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp
 35 40 45

Leu Ala Leu Ile Tyr Trp Asn Asp Asp Lys Arg Tyr Ser Pro Ser Leu
 50 55 60

Arg Ser Arg Leu Thr Ile Thr Lys Asp Thr Ser Lys Asn Gln Val Val
 65 70 75 80

Leu Thr Ile Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys
 85 90 95

Ala His Ser His Tyr Asp Tyr Asp Trp Gly Ser Tyr Phe Asp Tyr Trp
 100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
 115 120

<210> 39
 <211> 24
 <212> PRT
 <213> Homo sapiens

<400> 39

Ile Thr Leu Lys Glu Ser Gly Pro Thr Leu Val Lys Pro Thr Gln Thr
 1 5 10 15

Leu Thr Leu Thr Cys Thr Phe Ser
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<210> 40
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 40

Gly Phe Ser Leu Ser Ala Gly Gly Val Gly Val Gly
 1 5 10

<210> 41
 <211> 14

<212> PRT
 <213> Homo sapiens

<400> 41

Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu Ala
 1 5 10

<210> 42
 <211> 16
 <212> PRT
 <213> Homo sapiens

<400> 42

Leu Ile Tyr Trp Asn Asp Asp Lys Arg Tyr Ser Pro Ser Leu Arg Ser
 1 5 10 15

<210> 43
 <211> 32
 <212> PRT
 <213> Homo sapiens

<400> 43

Arg Leu Thr Ile Thr Lys Asp Thr Ser Lys Asn Gln Val Val Leu Thr
 1 5 10 15

Ile Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala His
 20 25 30

<210> 44
 <211> 13
 <212> PRT
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<400> 44

Ser His Tyr Asp Tyr Asp Trp Gly Ser Tyr Phe Asp Tyr
 1 5 10

<210> 45
 <211> 12
 <212> PRT
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<400> 45

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
 1 5 10

<210> 46
 <211> 342
 <212> DNA
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<400> 46
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 gcctctggag tccagacag gttcagtggc agtgggtcag gcactgattt cacactgaac 240
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<210> 47
 <211> 114
 <212> PRT
 <213> Homo sapiens

<400> 47
 Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
 1 5 10 15
 Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu Asp Ser
 20 25 30
 Asp Asp Gly Asn Thr Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln
 35 40 45
 Ser Pro Gln Leu Leu Ile Tyr Thr Leu Ser Tyr Arg Ala Ser Gly Val
 50 55 60
 Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn
 65 70 75 80
 Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln
 85 90 95
 Arg Ile Glu Phe Pro Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile
 100 105 110

Lys Arg

<210> 48
 <211> 23
 <212> PRT
 <213> Homo sapiens
 <400> 48

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
 1 5 10 15

Glu Pro Ala Ser Ile Ser Cys
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<210> 49
 <211> 17
 <212> PRT
 <213> Homo sapiens

<400> 49

Arg Ser Ser Gln Ser Leu Leu Asp Ser Asp Asp Gly Asn Thr Tyr Leu
 1 5 10 15

Asp

<210> 50
 <211> 15
 <212> PRT
 <213> Homo sapiens

<400> 50

Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr
 1 5 10 15

<210> 51
 <211> 7
 <212> PRT
 <213> Homo sapiens

<400> 51

Thr Leu Ser Tyr Arg Ala Ser
 1 5

<210> 52
 <211> 32
 <212> PRT
 <213> Homo sapiens

<400> 52

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
 1 5 10 15

Leu Asn Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
 20 25 30

<210> 53

<211> 9
 <212> PRT
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<400> 53

Met Gln Arg Ile Glu Phe Pro Ile Thr
 1 5

<210> 54
 <211> 11
 <212> PRT
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<400> 54

Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg
 1 5 10

<210> 55
 <211> 360
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 tgcaaccgct cctcaagag tcgagttatc atatcagtag acacgtctaa gaaccagttc 240
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<210> 56
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 <212> PRT
 <213> Homo sapiens

<400> 56

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
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Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Ala
 20 25 30

Asn Tyr Tyr Trp Thr Trp Ile Arg Gln His Pro Gly Lys Gly Leu Glu
 35 40 45

Trp Ile Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Tyr Cys Asn Pro Ser
 50 55 60

Leu Lys Ser Arg Val Ile Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
 65 70 75 80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
 85 90 95

Cys Ala Arg Gly Tyr Asn Trp Asn Tyr Phe Asp Tyr Trp Gly Gln Gly
 100 105 110

Thr Leu Val Thr Val Ser Ser Ala
 115 120

<210> 57
 <211> 24
 <212> PRT
 <213> Homo sapiens

<400> 57

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
 1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val
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<210> 58
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 58

Gly Gly Ser Ile Ser Ser Ala Asn Tyr Tyr Trp Thr
 1 5 10

<210> 59
 <211> 14
 <212> PRT
 <213> Homo sapiens

<400> 59

Trp Ile Arg Gln His Pro Gly Lys Gly Leu Glu Trp Ile Gly
 1 5 10

<210> 60
 <211> 16
 <212> PRT
 <213> Homo sapiens

<400> 60

Tyr Ile Tyr Tyr Ser Gly Ser Thr Tyr Cys Asn Pro Ser Leu Lys Ser
 1 5 10 15

<210> 61
 <211> 32
 <212> PRT
 <213> Homo sapiens

<400> 61

Arg Val Ile Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Lys
 1 5 10 15

Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg
 20 25 30

<210> 62
 <211> 9
 <212> PRT
 <213> Homo sapiens

<400> 62

Gly Tyr Asn Trp Asn Tyr Phe Asp Tyr
 1 5

<210> 63
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 63

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
 1 5 10

<210> 64
 <211> 330
 <212> DNA
 <213> Homo sapiens

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 aggttcagtg gcagtggtgc tgggacagag ttactctca ccatcagcag cctgcagttc 240
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 caagggacca aggtggaaat cgaacgaact 330

<210> 65
 <211> 109
 <212> PRT
 <213> Homo sapiens

<400> 65

Asp Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Asn
 20 25 30

Leu Ala Trp Tyr Gln Glu Arg Pro Gly Gln Ala Pro Arg Leu Leu Ile
 35 40 45

Tyr Gly Ala Ser Thr Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Ser
 65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Asn Lys Trp Pro Pro
 85 90 95

Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Glu Arg
 100 105

<210> 66
 <211> 23
 <212> PRT
 <213> Homo sapiens

<400> 66

Asp Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys
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<210> 67
 <211> 11
 <212> PRT
 <213> Homo sapiens

<400> 67

Arg Ala Ser Gln Ser Val Ser Ser Asn Leu Ala
 1 5 10

<210> 68
<211> 15
<212> PRT
<213> Homo sapiens

<400> 68

Trp Tyr Gln Glu Arg Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
1 5 10 15

<210> 69
<211> 7
<212> PRT
<213> Homo sapiens

<400> 69

Gly Ala Ser Thr Arg Ala Thr
1 5

<210> 70
<211> 32
<212> PRT
<213> Homo sapiens

<400> 70

Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr
1 5 10 15

Leu Thr Ile Ser Ser Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys
20 25 30

<210> 71
<211> 10
<212> PRT
<213> Homo sapiens

<400> 71

Gln Gln Tyr Asn Lys Trp Pro Pro Trp Thr
1 5 10

<210> 72
<211> 11
<212> PRT
<213> Homo sapiens

<400> 72

Phe Gly Gln Gly Thr Lys Val Glu Ile Glu Arg
1 5 10

<210> 73
<211> 378

<212> DNA

<213> Homo sapiens

<400> 73

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aaggggctgg agtgggtggc agttatatca tatgatggaa ataataaata ctatgcagac      180
tccgtgaagg gccgattcac catctccaga gacaattcca agaacacgct gtatctgcaa      240
atgaacagcc tgagagctga ggacacggct gtgtattact gtgcgagaga tctagtgggt      300
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<210> 74

<211> 126

<212> PRT

<213> Homo sapiens

<400> 74

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Arg Leu Ser Cys Ala Ala Ser Gly Phe Ala Phe Ser Ser Tyr Gly Met
                20              25              30

His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Val
        35              40              45

Ile Ser Tyr Asp Gly Asn Asn Lys Tyr Tyr Ala Asp Ser Val Lys Gly
        50              55              60

Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln
65              70              75              80

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
                85              90              95

Asp Leu Val Val Arg Gly Ile Arg Gly Tyr Tyr Tyr Tyr Phe Gly Met
        100              105              110

Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala
        115              120              125

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<210> 75

<211> 23

<212> PRT

<213> Homo sapiens

<400> 75

Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser Leu
1 5 10 15

Arg Leu Ser Cys Ala Ala Ser
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<210> 76

<211> 10

<212> PRT

<213> Homo sapiens

<400> 76

Gly Phe Ala Phe Ser Ser Tyr Gly Met His
1 5 10

<210> 77

<211> 14

<212> PRT

<213> Homo sapiens

<400> 77

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala
1 5 10

<210> 78

<211> 17

<212> PRT

<213> Homo sapiens

<400> 78

Val Ile Ser Tyr Asp Gly Asn Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 79

<211> 32

<212> PRT

<213> Homo sapiens

<400> 79

Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg

20 25 30

<210> 80
 <211> 18
 <212> PRT
 <213> Homo sapiens

<400> 80

Asp Leu Val Val Arg Gly Ile Arg Gly Tyr Tyr Tyr Tyr Phe Gly Met
 1 5 10 15

Asp Val

<210> 81
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 81

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala
 1 5 10

<210> 82
 <211> 339
 <212> DNA
 <213> Homo sapiens

<400> 82

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tacctgcaga agccagggca gtctccacag ctctgatct atttgggttc taatcggggc	180
tccgggggtcc ctgacaggtt cagtggcagt ggatcaggca cagattttac actgaaaatc	240
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atcaccttcg gccaaaggac acgactggag attaaacga	339

<210> 83
 <211> 113
 <212> PRT
 <213> Homo sapiens

<400> 83

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
 1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
 20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
 50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Gly
 85 90 95

Leu Gln Thr Pro Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys
 100 105 110

Arg

<210> 84
 <211> 23
 <212> PRT
 <213> Homo sapiens

<400> 84

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
 1 5 10 15

Glu Pro Ala Ser Ile Ser Cys
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<210> 85
 <211> 16
 <212> PRT
 <213> Homo sapiens

<400> 85

Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp
 1 5 10 15

<210> 86
 <211> 15
 <212> PRT
 <213> Homo sapiens

<400> 86

Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr
 1 5 10 15

<210> 87
 <211> 7
 <212> PRT
 <213> Homo sapiens

<400> 87

Leu Gly Ser Asn Arg Ala Ser
 1 5

<210> 88
 <211> 32
 <212> PRT
 <213> Homo sapiens

<400> 88

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr
 1 5 10 15

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
 20 25 30

<210> 89
 <211> 9
 <212> PRT
 <213> Homo sapiens

<400> 89

Met Gln Gly Leu Gln Thr Pro Ile Thr
 1 5

<210> 90
 <211> 11
 <212> PRT
 <213> Homo sapiens

<400> 90

Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg
 1 5 10

<210> 91
 <211> 366
 <212> DNA
 <213> Homo sapiens

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gcagactccg tgaagggccg attcaccatc tccagagaca attccaagaa cacgctgtat 240
 ctgcaaatga acagcctgag agccgaggac gcggctgtgt attactgtgc gagagatccc 300
 tttgactatg gtgactcctt ctttgactac tggggccagg gcaccctggt caccgtctcc 360
 tcagcc 366

<210> 92
 <211> 122
 <212> PRT
 <213> Homo sapiens

<400> 92

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
 20 25 30

Gly Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Val Ile Trp Phe Asp Gly Arg Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Ala Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Asp Pro Phe Asp Tyr Gly Asp Ser Phe Phe Asp Tyr Trp Gly
 100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala
 115 120

<210> 93
 <211> 25
 <212> PRT
 <213> Homo sapiens

<400> 93

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser

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25

<210> 94
 <211> 10
 <212> PRT
 <213> Homo sapiens

<400> 94

Gly Phe Thr Phe Ser Asn Tyr Gly Ile His
 1 5 10

<210> 95
 <211> 14
 <212> PRT
 <213> Homo sapiens

<400> 95

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala
 1 5 10

<210> 96
 <211> 17
 <212> PRT
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<400> 96

Val Ile Trp Phe Asp Gly Arg Asn Lys Tyr Tyr Ala Asp Ser Val Lys
 1 5 10 15

Gly

<210> 97
 <211> 32
 <212> PRT
 <213> Homo sapiens

<400> 97

Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln
 1 5 10 15

Met Asn Ser Leu Arg Ala Glu Asp Ala Ala Val Tyr Tyr Cys Ala Arg
 20 25 30

<210> 98
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 98

Asp Pro Phe Asp Tyr Gly Asp Ser Phe Phe Asp Tyr
 1 5 10

<210> 99
 <211> 12
 <212> PRT
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<400> 99

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
 1 5 10

<210> 100
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 <212> DNA
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<210> 101
 <211> 105
 <212> PRT
 <213> Homo sapiens

<400> 101

Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Arg Asp Arg Val
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Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr Leu Ala Trp
 20 25 30

Tyr Gln Gln Lys Pro Gly Lys Val Pro Asn Leu Leu Ile Tyr Ala Ala
 35 40 45

Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser
 50 55 60

Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Val
 65 70 75 80

Ala Thr Tyr Tyr Cys Gln Lys Tyr Asn Ser Ala Pro Leu Thr Phe Gly
85 90 95

Gly Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 102
<211> 20
<212> PRT
<213> Homo sapiens

<400> 102

Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Arg Asp Arg Val
1 5 10 15

Thr Ile Thr Cys
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<210> 103
<211> 11
<212> PRT
<213> Homo sapiens

<400> 103

Arg Ala Ser Gln Asp Ile Ser Asn Tyr Leu Ala
1 5 10

<210> 104
<211> 15
<212> PRT
<213> Homo sapiens

<400> 104

Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Asn Leu Leu Ile Tyr
1 5 10 15

<210> 105
<211> 6
<212> PRT
<213> Homo sapiens

<400> 105

Ala Ala Ser Thr Leu Gln
1 5

<210> 106
<211> 32
<212> PRT
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<400> 106

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
 1 5 10 15

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Val Ala Thr Tyr Tyr Cys
 20 25 30

<210> 107

<211> 9

<212> PRT

<213> Homo sapiens

<400> 107

Gln Lys Tyr Asn Ser Ala Pro Leu Thr
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<210> 108

<211> 11

<212> PRT

<213> Homo sapiens

<400> 108

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
 1 5 10

<210> 109

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<213> Homo sapiens

<400> 110

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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Tyr Met Thr Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ile Ser Gly Ser Ile Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Met Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Gly Ala Ala Ala Gly Thr Asp Ala Phe Asp Ile Trp Gly
100 105 110

His Gly Thr Lys Val Thr Val Ser Ser Ala
115 120

<210> 111
<211> 25
<212> PRT
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<400> 111

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser
20 25

<210> 112
<211> 10
<212> PRT
<213> Homo sapiens

<400> 112

Gly Phe Thr Phe Ser Asp Tyr Tyr Met Thr
1 5 10

<210> 113
<211> 14
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<213> Homo sapiens

<400> 113

Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
1 5 10

<210> 114

<211> 17

<212> PRT

<213> Homo sapiens

<400> 114

Tyr Ile Ser Ile Ser Gly Ser Ile Thr His Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 115

<211> 32

<212> PRT

<213> Homo sapiens

<400> 115

Arg Phe Thr Met Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 116

<211> 12

<212> PRT

<213> Homo sapiens

<400> 116

Asp Gly Ala Ala Ala Gly Thr Asp Ala Phe Asp Ile
1 5 10

<210> 117

<211> 12

<212> PRT

<213> Homo sapiens

<400> 117

Trp Gly His Gly Thr Lys Val Thr Val Ser Ser Ala
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<210> 118

<211> 321
 <212> DNA
 <213> Homo sapiens

<400> 118
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 ggccaggctc ccaggctcct catcttttgg gcattccacca gggccactgg tatcccagcc 180
 aggttcagtg gcagtgggtc tgggacagag ttcaactctca ccatcagcag cctacagtct 240
 gaagattttg cagtttatta ctgtcagcag tatcattact ggcccaacttt cggccctggg 300
 accaaagtgg atatcaaagc a 321

<210> 119
 <211> 107
 <212> PRT
 <213> Homo sapiens

<400> 119

Glu Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
 1 5 10 15

Asp Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Asn Val Ser Ser Asn
 20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
 35 40 45

Phe Gly Ala Ser Thr Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Ser
 65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr His Tyr Trp Pro Thr
 85 90 95

Phe Gly Pro Gly Thr Lys Val Asp Ile Lys Arg
 100 105

<210> 120
 <211> 23
 <212> PRT
 <213> Homo sapiens

<400> 120

Glu Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly

1 5 10 15

Asp Arg Ala Thr Leu Ser Cys
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<210> 121
<211> 11
<212> PRT
<213> Homo sapiens

<400> 121

Arg Ala Ser Gln Asn Val Ser Ser Asn Leu Ala
1 5 10

<210> 122
<211> 15
<212> PRT
<213> Homo sapiens

<400> 122

Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Phe
1 5 10 15

<210> 123
<211> 7
<212> PRT
<213> Homo sapiens

<400> 123

Gly Ala Ser Thr Arg Ala Thr
1 5

<210> 124
<211> 32
<212> PRT
<213> Homo sapiens

<400> 124

Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr
1 5 10 15

Leu Thr Ile Ser Ser Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys
20 25 30

<210> 125
<211> 8
<212> PRT
<213> Homo sapiens

<400> 125

Gln Gln Tyr His Tyr Trp Pro Thr
1 5

<210> 126
<211> 11
<212> PRT
<213> Homo sapiens

<400> 126

Phe Gly Pro Gly Thr Lys Val Asp Ile Lys Arg
1 5 10

<210> 127
<211> 360
<212> DNA
<213> Homo sapiens

<400> 127
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aaggcttctg gatacacctt caccggcttc tatatgcact ggggtgcgaca gacccttgga 120
caagggcttg agtggatggg atggatcaac cctaacagtg gtggcacata ttatgtacag 180
aagtttcagg gcagggtcac catgaccagg gacacgtcca tcagcacagt ctacatggag 240
ctgagcaggt tgagatctga cgacacggcc gtatattact gtgcgagaga tgggtatagc 300
agtggagagg actggttcga cccctggggc caggggaaccc tggtcaccgt ctcctcagcc 360

<210> 128
<211> 120
<212> PRT
<213> Homo sapiens

<400> 128

Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val
1 5 10 15

Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Phe Tyr Met
20 25 30

His Trp Val Arg Gln Thr Pro Gly Gln Gly Leu Glu Trp Met Gly Trp
35 40 45

Ile Asn Pro Asn Ser Gly Gly Thr Tyr Tyr Val Gln Lys Phe Gln Gly
50 55 60

Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Val Tyr Met Glu
65 70 75 80

Gly

<210> 133
 <211> 32
 <212> PRT
 <213> Homo sapiens

<400> 133

Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Val Tyr Met Glu
 1 5 10 15

Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg
 20 25 30

<210> 134
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 134

Asp Gly Tyr Ser Ser Gly Glu Asp Trp Phe Asp Pro
 1 5 10

<210> 135
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 135

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
 1 5 10

<210> 136
 <211> 339
 <212> DNA
 <213> Homo sapiens

<400> 136
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 tacctgcaga ggccaggcca gcctccacag ctctgatct atgaagtttc caaccggttc 180
 tctggagtgc cagatagggt cagtggcagc gggtcaggga cagatttcac actgaaaatc 240
 agccgggtgg aggctgagga tgttgggggt tattactgca tgcaaagtat acaccttccg 300
 ctcactttcg gcggaggag caaggtggag atcaaacga 339

<210> 137

<211> 113
 <212> PRT
 <213> Homo sapiens

<400> 137

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
 1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu His Ser
 20 25 30

Gly Gly Lys Thr Tyr Leu Tyr Trp Tyr Leu Gln Arg Pro Gly Gln Pro
 35 40 45

Pro Gln Leu Leu Ile Tyr Glu Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ser
 85 90 95

Ile His Leu Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105 110

Arg

<210> 138
 <211> 23
 <212> PRT
 <213> Homo sapiens

<400> 138

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
 1 5 10 15

Gln Pro Ala Ser Ile Ser Cys
 20

<210> 139
 <211> 16
 <212> PRT
 <213> Homo sapiens

<400> 139

Lys Ser Ser Gln Ser Leu Leu His Ser Gly Gly Lys Thr Tyr Leu Tyr

38

<210> 145
 <211> 360
 <212> DNA
 <213> Homo sapiens

<400> 145
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 gcagactcag tgaagggccg attcaccatc tccagagaca acgccaagaa ctactgtat 240
 ctgcaaatga acagcctgag agccgaggac acggctgtgt attactgtgc gagagaggac 300
 tgggtgggag ctacctttga ctactggggc caggggaaccc tggtcaccgt ctctcagcc 360

<210> 146
 <211> 120
 <212> PRT
 <213> Homo sapiens

<400> 146
 Gln Val Gln Leu Glu Gln Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Phe Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Phe Ile Ser Ser Ser Ser Ser Tyr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg Glu Asp Trp Val Gly Ala Thr Phe Asp Tyr Trp Gly Gln Gly
 100 105 110
 Thr Leu Val Thr Val Ser Ser Ala
 115 120

<210> 147
 <211> 25

<212> PRT
<213> Homo sapiens

<400> 147

Gln Val Gln Leu Glu Gln Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Phe Ser Cys Ala Ala Ser
20 25

<210> 148
<211> 10
<212> PRT
<213> Homo sapiens

<400> 148

Gly Phe Thr Phe Ser Ser Tyr Ser Met Asn
1 5 10

<210> 149
<211> 14
<212> PRT
<213> Homo sapiens

<400> 149

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
1 5 10

<210> 150
<211> 17
<212> PRT
<213> Homo sapiens

<400> 150

Phe Ile Ser Ser Ser Ser Tyr Ile Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 151
<211> 32
<212> PRT
<213> Homo sapiens

<400> 151

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
 20 25 30

<210> 152
 <211> 10
 <212> PRT
 <213> Homo sapiens

<400> 152

Glu Asp Trp Val Gly Ala Thr Phe Asp Tyr
 1 5 10

<210> 153
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 153

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
 1 5 10

<210> 154
 <211> 324
 <212> DNA
 <213> Homo sapiens

<400> 154
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 atcacttgtc gggcgagtca gggcattagg aattatttag cctggatatca gcagaaacca 120
 gggaaagttc ctaagctcct gatctatgct gcttccgctt tgaaattagg ggtcccatct 180
 cggttcagtg gcagtggatc tgggacagat ttcactctca ccatcagcag cctgcagcct 240
 gaagatgttg caacttatta ctgtcaaaag tataacagtg ccccgatcac cttcggccaa 300
 gggacacgac tggacattaa acga 324

<210> 155
 <211> 108
 <212> PRT
 <213> Homo sapiens

<400> 155

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Tyr
 20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile

35 40 45
 Tyr Ala Ala Ser Ala Leu Lys Leu Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80
 Glu Asp Val Ala Thr Tyr Tyr Cys Gln Lys Tyr Asn Ser Ala Pro Ile
 85 90 95
 Thr Phe Gly Gln Gly Thr Arg Leu Asp Ile Lys Arg
 100 105

<210> 156
 <211> 23
 <212> PRT
 <213> Homo sapiens

<400> 156

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys
 20

<210> 157
 <211> 11
 <212> PRT
 <213> Homo sapiens

<400> 157

Arg Ala Ser Gln Gly Ile Arg Asn Tyr Leu Ala
 1 5 10

<210> 158
 <211> 15
 <212> PRT
 <213> Homo sapiens

<400> 158

Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile Tyr
 1 5 10 15

<210> 159
 <211> 7
 <212> PRT
 <213> Homo sapiens

<400> 159

Ala Ala Ser Ala Leu Lys Leu
1 5

<210> 160

<211> 32

<212> PRT

<213> Homo sapiens

<400> 160

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Val Ala Thr Tyr Tyr Cys
20 25 30

<210> 161

<211> 9

<212> PRT

<213> Homo sapiens

<400> 161

Gln Lys Tyr Asn Ser Ala Pro Ile Thr
1 5

<210> 162

<211> 11

<212> PRT

<213> Homo sapiens

<400> 162

Phe Gly Gln Gly Thr Arg Leu Asp Ile Lys Arg
1 5 10

<210> 163

<211> 396

<212> DNA

<213> Homo sapiens

<400> 163

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cagcaccag ggaagggcct ggagtggatt gggtacatct attacagtgg gaacacctac 180
tacaaccggt cctcaagag tcgagttacc atatcagttg acacgtctaa gaaccagttc 240
tccctgaaac tgagctctgt gactgccgcg gacacggccg tgtattactg tgcgagagac 300
tattactatg atactagtgg tttttcctac cgttacgact ggtactacgg tatggacgtc 360

tggggccaag ggaccacggt caccgtctcc tcagcc

396

<210> 164
 <211> 132
 <212> PRT
 <213> Homo sapiens

<400> 164

Gln Val Gln Leu Glu Gln Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
 1 5 10 15

Asn Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Gly
 20 25 30

Gly Tyr Phe Trp Ser Trp Ile Arg Gln His Pro Gly Lys Gly Leu Glu
 35 40 45

Trp Ile Gly Tyr Ile Tyr Tyr Ser Gly Asn Thr Tyr Tyr Asn Pro Ser
 50 55 60

Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
 65 70 75 80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
 85 90 95

Cys Ala Arg Asp Tyr Tyr Tyr Asp Thr Ser Gly Phe Ser Tyr Arg Tyr
 100 105 110

Asp Trp Tyr Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr
 115 120 125

Val Ser Ser Ala
 130

<210> 165
 <211> 25
 <212> PRT
 <213> Homo sapiens

<400> 165

Gln Val Gln Leu Glu Gln Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
 1 5 10 15

Asn Leu Ser Leu Thr Cys Thr Val Ser
 20 25

<210> 166
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 166

Gly Gly Ser Ile Ser Ser Gly Gly Tyr Phe Trp Ser
 1 5 10

<210> 167
 <211> 14
 <212> PRT
 <213> Homo sapiens

<400> 167

Trp Ile Arg Gln His Pro Gly Lys Gly Leu Glu Trp Ile Gly
 1 5 10

<210> 168
 <211> 16
 <212> PRT
 <213> Homo sapiens

<400> 168

Tyr Ile Tyr Tyr Ser Gly Asn Thr Tyr Tyr Asn Pro Ser Leu Lys Ser
 1 5 10 15

<210> 169
 <211> 32
 <212> PRT
 <213> Homo sapiens

<400> 169

Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Lys
 1 5 10 15

Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg
 20 25 30

<210> 170
 <211> 21
 <212> PRT
 <213> Homo sapiens

<400> 170

Asp Tyr Tyr Tyr Asp Thr Ser Gly Phe Ser Tyr Arg Tyr Asp Trp Tyr
 1 5 10 15

Tyr Gly Met Asp Val
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<210> 171
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 171

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala
 1 5 10

<210> 172
 <211> 321
 <212> DNA
 <213> Homo sapiens

<400> 172
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 atcacttgcc gggcaagtca gggcattaga aatgatttag gctggtatca gcagaaacca 120
 gggaaagccc ctaagcgct gatctatgct gcatccagtt tgcaaatgg ggtcccatca 180
 aggttcagcg gcagtggatc tgggacagaa ttcactctca caatcagcag cctgcagcct 240
 gaagattttg caacttatta ctgtctacaa cataatactt acccgcggtt cggccaaggg 300
 accaaggtgg aaatcaaagc a 321

<210> 173
 <211> 107
 <212> PRT
 <213> Homo sapiens

<400> 173

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
 20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
 35 40 45

Tyr Ala Ala Ser Ser Leu Gln Asn Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln His Asn Thr Tyr Pro Ala
 85 90 95

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 174
<211> 23
<212> PRT
<213> Homo sapiens

<400> 174

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys
20

<210> 175
<211> 11
<212> PRT
<213> Homo sapiens

<400> 175

Arg Ala Ser Gln Gly Ile Arg Asn Asp Leu Gly
1 5 10

<210> 176
<211> 15
<212> PRT
<213> Homo sapiens

<400> 176

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile Tyr
1 5 10 15

<210> 177
<211> 7
<212> PRT
<213> Homo sapiens

<400> 177

Ala Ala Ser Ser Leu Gln Asn
1 5

<210> 178
<211> 32
<212> PRT
<213> Homo sapiens

<400> 178

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr
 1 5 10 15

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys
 20 25 30

<210> 179
 <211> 8
 <212> PRT
 <213> Homo sapiens

<400> 179

Leu Gln His Asn Thr Tyr Pro Ala
 1 5

<210> 180
 <211> 11
 <212> PRT
 <213> Homo sapiens

<400> 180

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
 1 5 10

<210> 181
 <211> 354
 <212> DNA
 <213> Homo sapiens

<400> 181
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 caggggttctg gatacatctt taccaactac tggatcggct gggcgcgcca gatgcccggg 120
 aaaggcctgg agtggatggg ggtcatctat cctgatgact ctgataccag atacagcccg 180
 tccttccaag gccaggtcac catctcagcc gacaagtcca tcagcaccgc ctacctgcag 240
 tggagcagcc tgaaggcctc ggacaccgcc atatattact gtgcgagaca aaaatggcta 300
 caacaccctt ttgactactg gggccaggga accctgggtca ccgtctcctc agcc 354

<210> 182
 <211> 118
 <212> PRT
 <213> Homo sapiens

<400> 182

Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser Leu
 1 5 10 15

Lys Ile Ser Cys Gln Gly Ser Gly Tyr Ile Phe Thr Asn Tyr Trp Ile

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<210> 183
<211> 23
<212> PRT
<213> Homo sapiens
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Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser Leu
1 5 10 15

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<210> 184
<211> 10
<212> PRT
<213> Homo sapiens
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Gly Tyr Ile Phe Thr Asn Tyr Trp Ile Gly
1 5 10

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<210> 185
<211> 14
<212> PRT
<213> Homo sapiens
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Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly

1 5 10

<210> 186
<211> 17
<212> PRT
<213> Homo sapiens

<400> 186

Val Ile Tyr Pro Asp Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe Gln
1 5 10 15

Gly

<210> 187
<211> 32
<212> PRT
<213> Homo sapiens

<400> 187

Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu Gln
1 5 10 15

Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Ile Tyr Tyr Cys Ala Arg
20 25 30

<210> 188
<211> 10
<212> PRT
<213> Homo sapiens

<400> 188

Gln Lys Trp Leu Gln His Pro Phe Asp Tyr
1 5 10

<210> 189
<211> 12
<212> PRT
<213> Homo sapiens

<400> 189

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
1 5 10

<210> 190
<211> 327
<212> DNA
<213> Homo sapiens

<400> 190

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 cctggccagg ctcccaggct cctcatctat ggtgcatcca gcagggccac tggcatccca 180
 gacaggttca gtggcagtgg gtctgggaca gacttcactc tcaccatcag cagactggag 240
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 caagggacca aggtggaaat caaacga 327

<210> 191
 <211> 109
 <212> PRT
 <213> Homo sapiens

<400> 191

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

Glu Arg Val Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Arg
 20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
 35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
 65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
 85 90 95

Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
 100 105

<210> 192
 <211> 23
 <212> PRT
 <213> Homo sapiens

<400> 192

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

Glu Arg Val Thr Leu Ser Cys
 20

<210> 193
<211> 12
<212> PRT
<213> Homo sapiens

<400> 193

Arg Ala Ser Gln Ser Val Ser Ser Arg Tyr Leu Ala
1 5 10

<210> 194
<211> 15
<212> PRT
<213> Homo sapiens

<400> 194

Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
1 5 10 15

<210> 195
<211> 7
<212> PRT
<213> Homo sapiens

<400> 195

Gly Ala Ser Ser Arg Ala Thr
1 5

<210> 196
<211> 31
<212> PRT
<213> Homo sapiens

<400> 196

Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Leu Thr Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr
20 25 30

<210> 197
<211> 9
<212> PRT
<213> Homo sapiens

<400> 197

Gln Gln Tyr Gly Ser Ser Pro Arg Thr
1 5

<210> 198
 <211> 11
 <212> PRT
 <213> Homo sapiens

<400> 198

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
 1 5 10

<210> 199
 <211> 369
 <212> DNA
 <213> Homo sapiens

<400> 199
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 cctggacaag ggcttgagtg gatgggatgg atcaacccta acagtgggtg cacaaactat 180
 gcacagaagt ttcaggacag ggtcaccatg accagggaca cgtccatcag cacagcctac 240
 atggagctga gcaggctgag atctgacgac acggccgtgt attactgtgc gagagatttc 300
 tttggttcgg ggagtctcct ctactttgac tactggggcc agggaaacct ggtcaccgtc 360
 tcctcagcc 369

<210> 200
 <211> 123
 <212> PRT
 <213> Homo sapiens

<400> 200

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
 20 25 30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
 50 55 60

Gln Asp Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
 65 70 75 80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Asp Phe Phe Gly Ser Gly Ser Leu Leu Tyr Phe Asp Tyr Trp
 100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
 115 120

<210> 201
 <211> 25
 <212> PRT
 <213> Homo sapiens

<400> 201

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser
 20 25

<210> 202
 <211> 10
 <212> PRT
 <213> Homo sapiens

<400> 202

Gly Tyr Thr Phe Thr Gly Tyr Tyr Met His
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Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly
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<400> 204

Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe Gln
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Asp

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 <212> PRT
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<400> 205

Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr Met Glu
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Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg
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Asp Phe Phe Gly Ser Gly Ser Leu Leu Tyr Phe Asp Tyr
 1 5 10

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Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
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 tctggagtgc cagatagggt cagtggcagc gggtcaggga cagatttcac actgaaaatc 240
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<400> 209

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
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Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu His Ser
 20 25 30

Gly Gly Lys Thr Tyr Leu Tyr Trp Tyr Leu Gln Arg Pro Gly Gln Pro
 35 40 45

Pro Gln Leu Leu Ile Tyr Glu Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ser
 85 90 95

Ile His Leu Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105 110

Arg

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 <212> PRT
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<400> 210

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
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Gln Pro Ala Ser Ile Ser Cys
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<400> 211

Lys Ser Ser Gln Ser Leu Leu His Ser Gly Gly Lys Thr Tyr Leu Tyr
 1 5 10 15

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Trp Tyr Leu Gln Arg Pro Gly Gln Pro Pro Gln Leu Leu Ile Tyr
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<400> 213

Glu Val Ser Asn Arg Phe Ser
 1 5

<210> 214
 <211> 32
 <212> PRT
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<400> 214

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
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Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
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<400> 215

Met Gln Ser Ile His Leu Pro Leu Thr
 1 5

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Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
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<212> DNA

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<400> 217

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ccaggcaagg ggctggagtg ggtggcagtt atatggtatg atggaagtaa taaatactat      180
gcagactccg tgaagggccg attcaccatc tccagagaca attccaagaa cagcgtgtat      240
ctgcaaatga acagcctgag agccgaggac acggctgtgt attactgtgc gaaagatgag      300
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Gln Val Gln Leu Glu Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Asn Tyr
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Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
          35           40           45

Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
          50           55           60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
          65           70           75           80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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Ala Lys Asp Glu Glu Tyr Tyr Tyr Val Ser Gly Leu Asp Tyr Trp Gly
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Gln Gly Thr Leu Val Thr Val Ser Ser Ala
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<211> 25

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Gln Val Gln Leu Glu Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
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Ser Leu Arg Leu Ser Cys Ala Ala Ser
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Gly Phe Thr Phe Asn Asn Tyr Gly Met His
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<400> 221

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala
1 5 10

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<211> 17

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<213> Homo sapiens

<400> 222

Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
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Gly

<210> 223

<211> 32

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<213> Homo sapiens

<400> 223

Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln
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Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys

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25

30

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Asp Glu Glu Tyr Tyr Tyr Val Ser Gly Leu Asp Tyr
 1 5 10

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Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
 1 5 10

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 ggcagtggat ctgggacaga ttctactctc accatcagca gcctgcagcc tgaagatggt 240
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 gtggagatca aacga 315

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<400> 227

Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Arg Asp Arg Val
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Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr Leu Ala Trp
 20 25 30

Tyr Gln Gln Lys Pro Gly Lys Val Pro Asn Leu Leu Ile Tyr Ala Ala
 35 40 45

Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser
 50 55 60

Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Val
 65 70 75 80

Ala Thr Tyr Tyr Cys Gln Lys Tyr Asn Ser Ala Pro Leu Thr Phe Gly
 85 90 95

Gly Gly Thr Lys Val Glu Ile Lys Arg
 100 105

<210> 228
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 <213> Homo sapiens

<400> 228

Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Arg Asp Arg Val
 1 5 10 15

Thr Ile Thr Cys
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<400> 229

Arg Ala Ser Gln Asp Ile Ser Asn Tyr Leu Ala
 1 5 10

<210> 230
 <211> 15
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<400> 230

Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Asn Leu Leu Ile Tyr
 1 5 10 15

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<400> 231

Ala Ala Ser Thr Leu Gln
1 5

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<400> 232

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
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Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Val Ala Thr Tyr Tyr Cys
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Gln Lys Tyr Asn Ser Ala Pro Leu Thr
1 5

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<400> 234

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
1 5 10

<210> 235
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cagccccag gaaaggccct ggactggctt aactcattt attggaatga tgataagcac 180
tacagcccat ctctgaagag caggcttacc ataccaagg acacctcaa aaaccagggtg 240
gtccttagaa tgaccaacat ggacctgtg gacacagcca cttattactg tgcacacctg 300
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gtctcctcag cc 372

<210> 236
 <211> 124
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 <213> Homo sapiens

<400> 236

Gln Ile Thr Leu Lys Glu Ser Gly Pro Thr Leu Val Thr Pro Thr Gln
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Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Gly
 20 25 30

Gly Met Gly Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Asp
 35 40 45

Trp Leu Thr Leu Ile Tyr Trp Asn Asp Asp Lys His Tyr Ser Pro Ser
 50 55 60

Leu Lys Ser Arg Leu Thr Ile Thr Lys Asp Thr Ser Lys Asn Gln Val
 65 70 75 80

Val Leu Arg Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
 85 90 95

Cys Ala His Leu His Tyr Asp Ile Leu Thr Gly Phe Asn Phe Asp Tyr
 100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
 115 120

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<400> 237

Gln Ile Thr Leu Lys Glu Ser Gly Pro Thr Leu Val Thr Pro Thr Gln
 1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser
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<400> 238

Gly Phe Ser Leu Ser Thr Gly Gly Met Gly Val Gly
 1 5 10

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<400> 239

Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Asp Trp Leu Thr
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<400> 240

Leu Ile Tyr Trp Asn Asp Asp Lys His Tyr Ser Pro Ser Leu Lys Ser
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<210> 241

<211> 32

<212> PRT

<213> Homo sapiens

<400> 241

Arg Leu Thr Ile Thr Lys Asp Thr Ser Lys Asn Gln Val Val Leu Arg
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Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala His
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Leu His Tyr Asp Ile Leu Thr Gly Phe Asn Phe Asp Tyr
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Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
 1 5 10

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<213> Homo sapiens

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<211> 114

<212> PRT

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Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
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Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu Asp Ser
 20 25 30

Asp Asp Gly Asn Thr Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln
 35 40 45

Ser Pro Gln Leu Leu Ile Tyr Thr Leu Ser Tyr Arg Ala Ser Gly Val
 50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys
 65 70 75 80

Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln
 85 90 95

Arg Ile Glu Phe Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
 100 105 110

Lys Arg

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<400> 246

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
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Glu Pro Ala Ser Ile Ser Cys
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<212> PRT
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<400> 247

Arg Ser Ser Gln Ser Leu Leu Asp Ser Asp Asp Gly Asn Thr Tyr Leu
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Asp

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<400> 248

Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr
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<400> 249

Thr Leu Ser Tyr Arg Ala Ser
1 5

<210> 250
<211> 32
<212> PRT
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<400> 250

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
 1 5 10 15

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
 20 25 30

<210> 251
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 <213> Homo sapiens

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Met Gln Arg Ile Glu Phe Pro Leu Thr
 1 5

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Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
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<400> 253

Xaa Ser Gly Pro Gly Leu Val Lys Pro Ser Gln Xaa Leu Ser Leu Thr
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Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Xaa Xaa Tyr Xaa Trp Xaa
 20 25 30

Trp Ile Arg Xaa His Pro Gly Lys Gly Leu Glu Trp Ile Gly Tyr Ile
 35 40 45

Tyr Tyr Ser Gly Xaa Thr Tyr Xaa Asn Pro Ser Leu Lys Ser Arg Val
 50 55 60

Xaa Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Xaa Leu Ser
 65 70 75 80

Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg
 - 85 90

<210> 254
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 <223> Xaa is Y or F

<220>
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 <223> Xaa is T or S

<400> 254

Gly Gly Ser Ile Ser Ser Xaa Xaa Tyr Xaa Trp Xaa
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<210> 255
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 <223> Xaa is S or N

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 <223> Xaa is C, S, or Y

<400> 255

Tyr Ile Tyr Tyr Ser Gly Xaa Thr Tyr Xaa Asn Pro Ser Leu Lys Ser
 1 5 10 15

<210> 256
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 <223> Xaa is D or G

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<400> 256

Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val
 1 5 10 15

Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Xaa Tyr Met
 20 25 30

His Trp Val Arg Gln Xaa Pro Gly Gln Gly Leu Glu Trp Met Gly Trp
 35 40 45

Ile Asn Pro Asn Ser Gly Gly Thr Xaa Tyr Xaa Gln Lys Phe Gln Xaa
 50 55 60

Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Xaa Tyr Met Glu
 65 70 75 80

Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg
 85 90 95

<210> 257
 <211> 10
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<213> Homo sapiens

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<223> Xaa is Y or F

<400> 257

Gly	Tyr	Thr	Phe	Thr	Gly	Xaa	Tyr	Met	His
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<210> 258

<211> 17

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<223> Xaa is D or G

<400> 258

Trp	Ile	Asn	Pro	Asn	Ser	Gly	Gly	Thr	Xaa	Tyr	Xaa	Gln	Lys	Phe	Gln
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Xaa

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Xaa Xaa Xaa Gly Ser Gly Ser Xaa Xaa
1 5

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<222> (31)..(31)

<223> Xaa is S or G

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<221> VARIANT

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<223> Xaa is A or T

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<400> 260

Ile Thr Leu Lys Glu Ser Gly Pro Thr Leu Val Xaa Pro Thr Gln Thr
 1 5 10 15

Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Xaa Xaa Gly
 20 25 30

Xaa Gly Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Xaa Trp
 35 40 45

Leu Xaa Leu Ile Tyr Trp Asn Asp Asp Lys Xaa Tyr Ser Pro Ser Leu
 50 55 60

Xaa Ser Arg Leu Thr Ile Thr Lys Asp Thr Ser Lys Asn Gln Val Val
 65 70 75 80

Leu Xaa Xaa Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys
 85 90 95

Ala His

<210> 261
 <211> 12
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<220>
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 <223> Xaa is M or V

<400> 261

Gly Phe Ser Leu Ser Xaa Xaa Gly Xaa Gly Val Gly
 1 5 10

<210> 262
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<400> 262

Leu Ile Tyr Trp Asn Asp Asp Lys Xaa Tyr Ser Pro Ser Leu Xaa Ser
 1 5 10 15

<210> 263
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 <212> PRT
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<223> Xaa is Y or N

<400> 263

Xaa Tyr Asp Ile Leu Thr Gly Xaa Xaa
1 5

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<211> 7

<212> PRT

<213> Homo sapiens

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<400> 264

Tyr Asp Tyr Xaa Trp Gly Ser
1 5

<210> 265

<211> 98

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<213> Homo sapiens

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<223> Xaa is E or Q

<220>

<221> VARIANT

<222> (30)..(30)

<223> Xaa is S or N

<220>

<221> VARIANT

<222> (31)..(31)

<223> Xaa is S or N

<220>

<221> VARIANT

<222> (34)..(34)

<223> Xaa is M or I

<220>

<221> VARIANT

<222> (53)..(53)

<223> Xaa is Y or F

<220>

<221> VARIANT

<222> (56)..(56)

<223> Xaa is S or R

<220>

<221> VARIANT

<222> (91)..(91)

<223> Xaa is T or A

<220>

<221> VARIANT

<222> (98)..(98)

<223> Xaa is R or K

<400> 265

Gln Val Gln Leu Xaa Xaa Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Xaa Xaa Tyr
20 25 30

Gly Xaa His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Trp Xaa Asp Gly Xaa Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Xaa Ala Val Tyr Tyr Cys
85 90 95

Ala Xaa

<210> 266

<211> 10

<212> PRT

<213> Homo sapiens

<220>

<221> VARIANT

<222> (5)..(5)

<223> Xaa is S or N

<220>
 <221> VARIANT
 <222> (6)..(6)
 <223> Xaa is S or N

<220>
 <221> VARIANT
 <222> (9)..(9)
 <223> Xaa is M or I

<400> 266

Gly Phe Thr Phe Xaa Xaa Tyr Gly Xaa His
 1 5 10

<210> 267
 <211> 17
 <212> PRT
 <213> Homo sapiens

<220>
 <221> VARIANT
 <222> (4)..(4)
 <223> Xaa is Y or F

<220>
 <221> VARIANT
 <222> (7)..(7)
 <223> Xaa is S or R

<400> 267

Val Ile Trp Xaa Asp Gly Xaa Asn Lys Tyr Tyr Ala Asp Ser Val Lys
 1 5 10 15

Gly

<210> 268
 <211> 7
 <212> PRT
 <213> Homo sapiens

<220>
 <221> VARIANT
 <222> (7)..(7)
 <223> Xaa is S or L

<400> 268

Tyr Tyr Tyr Gly Ser Gly Xaa
 1 5

<210> 269
<211> 5
<212> PRT
<213> Homo sapiens

<220>
<221> VARIANT
<222> (5)..(5)
<223> Xaa is Y or S

<400> 269

Asp Tyr Gly Asp Xaa
1 5

<210> 270
<211> 98
<212> PRT
<213> Homo sapiens

<220>
<221> VARIANT
<222> (31)..(31)
<223> Xaa is D or S

<220>
<221> VARIANT
<222> (33)..(33)
<223> Xaa is S or Y

<220>
<221> VARIANT
<222> (35)..(35)
<223> Xaa is S or T

<220>
<221> VARIANT
<222> (53)..(53)
<223> Xaa is S or I

<220>
<221> VARIANT
<222> (57)..(57)
<223> Xaa is T or I

<220>
<221> VARIANT
<222> (58)..(58)
<223> Xaa is T or I

<220>
<221> VARIANT
<222> (59)..(59)
<223> Xaa is Y or H

<220>
 <221> VARIANT
 <222> (70)..(70)
 <223> Xaa is I or M

<400> 270

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Xaa Tyr
 20 25 30

Xaa Met Xaa Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Tyr Ile Ser Xaa Ser Gly Ser Xaa Xaa Xaa Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Xaa Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 271
 <211> 10
 <212> PRT
 <213> Homo sapiens

<220>
 <221> VARIANT
 <222> (6)..(6)
 <223> Xaa is D or S

<220>
 <221> VARIANT
 <222> (8)..(8)
 <223> Xaa is S or Y

<220>
 <221> VARIANT
 <222> (10)..(10)
 <223> Xaa is S or T

<400> 271

Gly Phe Thr Phe Ser Xaa Tyr Xaa Met Xaa
1 5 10

<210> 272

<211> 17

<212> PRT

<213> Homo sapiens

<220>

<221> VARIANT

<222> (4)..(4)

<223> Xaa is S or I

<220>

<221> VARIANT

<222> (8)..(8)

<223> Xaa is T or I

<220>

<221> VARIANT

<222> (9)..(9)

<223> Xaa is T or I

<220>

<221> VARIANT

<222> (10)..(10)

<223> Xaa is Y or H

<400> 272

Tyr Ile Ser Xaa Ser Gly Ser Xaa Xaa Xaa Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 273

<211> 10

<212> PRT

<213> Homo sapiens

<220>

<221> VARIANT

<222> (1)..(1)

<223> Xaa is G or D

<220>

<221> VARIANT

<222> (2)..(2)

<223> Xaa is I or G

<400> 273

Xaa Xaa Ala Ala Ala Gly Ala Phe Asp Ile
 1 5 10

<210> 274
 <211> 98
 <212> PRT
 <213> Homo sapiens

<220>
 <221> VARIANT
 <222> (1)..(1)
 <223> Xaa is E or Q

<220>
 <221> VARIANT
 <222> (5)..(5)
 <223> Xaa is V or E

<220>
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 <222> (6)..(6)
 <223> Xaa is E or Q

<220>
 <221> VARIANT
 <222> (20)..(20)
 <223> Xaa is F or L

<220>
 <221> VARIANT
 <222> (50)..(50)
 <223> Xaa is S or F

<400> 274

Xaa Val Gln Leu Xaa Xaa Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15

Ser Leu Arg Xaa Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Xaa Ile Ser Ser Ser Ser Ser Tyr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys

85

90

95

Ala Arg

<210> 275
<211> 17
<212> PRT
<213> Homo sapiens

<220>
<221> VARIANT
<222> (1)..(1)
<223> Xaa is S or F

<400> 275

Xaa	Ile	Ser	Ser	Ser	Ser	Ser	Tyr	Ile	Tyr	Tyr	Ala	Asp	Ser	Val	Lys
1				5					10					15	

Gly

<210> 276
<211> 9
<212> PRT
<213> Homo sapiens

<220>
<221> VARIANT
<222> (1)..(1)
<223> Xaa is G or D

<220>
<221> VARIANT
<222> (2)..(2)
<223> Xaa is I or W

<400> 276

Xaa	Xaa	Val	Gly	Ala	Thr	Phe	Asp	Tyr
1					5			

<210> 277
<211> 96
<212> PRT
<213> Homo sapiens

<220>
<221> VARIANT
<222> (26)..(26)
<223> Xaa is T or A

<220>
 <221> VARIANT
 <222> (54)..(54)
 <223> Xaa is S or N

<400> 277

Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser Leu
 1 5 10 15

Arg Leu Ser Cys Ala Ala Ser Gly Phe Xaa Phe Ser Ser Tyr Gly Met
 20 25 30

His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Val
 35 40 45

Ile Ser Tyr Asp Gly Xaa Asn Lys Tyr Tyr Ala Asp Ser Val Lys Gly
 50 55 60

Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln
 65 70 75 80

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys
 85 90 95

<210> 278
 <211> 10
 <212> PRT
 <213> Homo sapiens

<220>
 <221> VARIANT
 <222> (3)..(3)
 <223> Xaa is T or A

<400> 278

Gly Phe Xaa Phe Ser Ser Tyr Gly Met His
 1 5 10

<210> 279
 <211> 17
 <212> PRT
 <213> Homo sapiens

<220>
 <221> VARIANT

<222> (7)..(7)
<223> Xaa is S or N

<400> 279

Val	Ile	Ser	Tyr	Asp	Gly	Xaa	Asn	Lys	Tyr	Tyr	Ala	Asp	Ser	Val	Lys
1					5				10					15	

Gly

<210> 280
<211> 9
<212> PRT
<213> Homo sapiens

<220>
<221> VARIANT
<222> (1)..(1)
<223> Xaa is I or D

<220>
<221> VARIANT
<222> (2)..(2)
<223> Xaa is T or L

<220>
<221> VARIANT
<222> (3)..(3)
<223> Xaa is M or V

<220>
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<222> (7)..(7)
<223> Xaa is V or I

<220>
<221> VARIANT
<222> (8)..(8)
<223> Xaa is I or R

<220>
<221> VARIANT
<222> (9)..(9)
<223> Xaa is I or G

<400> 280

Xaa	Xaa	Xaa	Val	Arg	Gly	Xaa	Xaa	Xaa
1					5			

<210> 281
<211> 97
<212> PRT
<213> Homo sapiens

<220>
 <221> VARIANT
 <222> (27)..(27)
 <223> Xaa is G or D

<220>
 <221> VARIANT
 <222> (31)..(31)
 <223> Xaa is S or N

<220>
 <221> VARIANT
 <222> (51)..(51)
 <223> Xaa is I or F

<400> 281

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
 1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Xaa Ser Ile Ser Xaa Tyr
 20 25 30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
 35 40 45

Gly Tyr Xaa Tyr Tyr Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
 50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
 65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95

Arg

<210> 282
 <211> 10
 <212> PRT
 <213> Homo sapiens

<220>
 <221> VARIANT
 <222> (2)..(2)
 <223> Xaa is G or D

<220>
 <221> VARIANT

<222> (6)..(6)
<223> Xaa is S or N

<400> 282

Gly Xaa Ser Ile Ser Xaa Tyr Tyr Trp Ser
1 5 10

<210> 283
<211> 16
<212> PRT
<213> Homo sapiens

<220>
<221> VARIANT
<222> (2)..(2)
<223> Xaa is I or F

<400> 283

Tyr Xaa Tyr Tyr Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys Ser
1 5 10 15

<210> 284
<211> 6
<212> PRT
<213> Homo sapiens

<220>
<221> VARIANT
<222> (1)..(1)
<223> Xaa is S or D

<220>
<221> VARIANT
<222> (2)..(2)
<223> Xaa is S or R

<400> 284

Xaa Xaa Gly Trp Asp Tyr
1 5

<210> 285
<211> 96
<212> PRT
<213> Homo sapiens

<220>
<221> VARIANT
<222> (21)..(21)
<223> Xaa is K or Q

<220>
 <221> VARIANT
 <222> (26)..(26)
 <223> Xaa is S or I

<220>
 <221> VARIANT
 <222> (29)..(29)
 <223> Xaa is S or N

<220>
 <221> VARIANT
 <222> (48)..(48)
 <223> Xaa is I or V

<220>
 <221> VARIANT
 <222> (52)..(52)
 <223> Xaa is G or D

<220>
 <221> VARIANT
 <222> (91)..(91)
 <223> Xaa is M or I

<400> 285

Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Glu	Ser	Leu
1				5					10					15	

Lys	Ile	Ser	Cys	Xaa	Gly	Ser	Gly	Tyr	Xaa	Phe	Thr	Xaa	Tyr	Trp	Ile
			20					25					30		

Gly	Trp	Val	Arg	Gln	Met	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Met	Gly	Xaa
		35					40					45			

Ile	Tyr	Pro	Xaa	Asp	Ser	Asp	Thr	Arg	Tyr	Ser	Pro	Ser	Phe	Gln	Gly
	50					55					60				

Gln	Val	Thr	Ile	Ser	Ala	Asp	Lys	Ser	Ile	Ser	Thr	Ala	Tyr	Leu	Gln
65					70					75				80	

Trp	Ser	Ser	Leu	Lys	Ala	Ser	Asp	Thr	Ala	Xaa	Tyr	Tyr	Cys	Ala	Arg
				85					90					95	

<210> 286
 <211> 10
 <212> PRT
 <213> Homo sapiens

<220>

<221> VARIANT
<222> (3)..(3)
<223> Xaa is S or I

<220>
<221> VARIANT
<222> (6)..(6)
<223> Xaa is S or N

<400> 286

Gly Tyr Xaa Phe Thr Xaa Tyr Trp Ile Gly
1 5 10

<210> 287
<211> 17
<212> PRT
<213> Homo sapiens

<220>
<221> VARIANT
<222> (1)..(1)
<223> Xaa is I or V

<220>
<221> VARIANT
<222> (5)..(5)
<223> Xaa is G or D

<400> 287

Xaa Ile Tyr Pro Xaa Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe Gln
1 5 10 15

Gly

<210> 288
<211> 8
<212> PRT
<213> Homo sapiens

<220>
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<222> (1)..(1)
<223> Xaa is R or K

<220>
<221> VARIANT
<222> (5)..(5)
<223> Xaa is L or H

<400> 288

Xaa Trp Leu Gln Xaa Phe Asp Tyr
 1 5

<210> 289

<211> 470

<212> PRT

<213> Homo sapiens

<400> 289

Lys Arg Phe His Asp Val Leu Gly Asn Glu Arg Pro Ser Ala Tyr Met
 1 5 10 15

Arg Glu His Asn Gln Leu Asn Gly Trp Ser Ser Asp Glu Asn Asp Trp
 20 25 30

Asn Glu Lys Leu Tyr Pro Val Trp Lys Arg Gly Asp Met Arg Trp Lys
 35 40 45

Asn Ser Trp Lys Gly Gly Arg Val Gln Ala Val Leu Thr Ser Asp Ser
 50 55 60

Pro Ala Leu Val Gly Ser Asn Ile Thr Phe Ala Val Asn Leu Ile Phe
 65 70 75 80

Pro Arg Cys Gln Lys Glu Asp Ala Asn Gly Asn Ile Val Tyr Glu Lys
 85 90 95

Asn Cys Arg Asn Glu Ala Gly Leu Ser Ala Asp Pro Tyr Val Tyr Asn
 100 105 110

Trp Thr Ala Trp Ser Glu Asp Ser Asp Gly Glu Asn Gly Thr Gly Gln
 115 120 125

Ser His His Asn Val Phe Pro Asp Gly Lys Pro Phe Pro His His Pro
 130 135 140

Gly Trp Arg Arg Trp Asn Phe Ile Tyr Val Phe His Thr Leu Gly Gln
 145 150 155 160

Tyr Phe Gln Lys Leu Gly Arg Cys Ser Val Arg Val Ser Val Asn Thr
 165 170 175

Ala Asn Val Thr Leu Gly Pro Gln Leu Met Glu Val Thr Val Tyr Arg
 180 185 190

Arg His Gly Arg Ala Tyr Val Pro Ile Ala Gln Val Lys Asp Val Tyr
195 200 205

Val Val Thr Asp Gln Ile Pro Val Phe Val Thr Met Phe Gln Lys Asn
210 215 220

Asp Arg Asn Ser Ser Asp Glu Thr Phe Leu Lys Asp Leu Pro Ile Met
225 230 235 240

Phe Asp Val Leu Ile His Asp Pro Ser His Phe Leu Asn Tyr Ser Thr
245 250 255

Ile Asn Tyr Lys Trp Ser Phe Gly Asp Asn Thr Gly Leu Phe Val Ser
260 265 270

Thr Asn His Thr Val Asn His Thr Tyr Val Leu Asn Gly Thr Phe Ser
275 280 285

Leu Asn Leu Thr Val Lys Ala Ala Ala Pro Gly Pro Cys Pro Pro Pro
290 295 300

Pro Pro Pro Pro Arg Pro Ser Lys Pro Thr Pro Ser Leu Ala Thr Thr
305 310 315 320

Leu Lys Ser Tyr Asp Ser Asn Thr Pro Gly Pro Ala Gly Asp Asn Pro
325 330 335

Leu Glu Leu Ser Arg Ile Pro Asp Glu Asn Cys Gln Ile Asn Arg Tyr
340 345 350

Gly His Phe Gln Ala Thr Ile Thr Ile Val Glu Gly Ile Leu Glu Val
355 360 365

Asn Ile Ile Gln Met Thr Asp Val Leu Met Pro Val Pro Trp Pro Glu
370 375 380

Ser Ser Leu Ile Asp Phe Val Val Thr Cys Gln Gly Ser Ile Pro Thr
385 390 395 400

Glu Val Cys Thr Ile Ile Ser Asp Pro Thr Cys Glu Ile Thr Gln Asn
405 410 415

Thr Val Cys Ser Pro Val Asp Val Asp Glu Met Cys Leu Leu Thr Val
420 425 430

Arg Arg Thr Phe Asn Gly Ser Gly Thr Tyr Cys Val Asn Leu Thr Leu
 435 440 445

Gly Asp Asp Thr Ser Leu Ala Leu Thr Ser Thr Leu Ile Ser Val Pro
 450 455 460

Asp Arg Asp Pro Ala Ser
 465 470

<210> 290
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> chemically synthesized primer

<400> 290
 caccatggac tggcacctgg aggatc

26

<210> 291
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> chemically synthesized primer

<400> 291
 caccatggac tggacctgga gacatc

26

<210> 292
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> chemically synthesized primer

<400> 292
 caccatggac tggacctgga gggtc

25

<210> 293
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> chemically synthesized primer

<400> 293
 caccatggac tggatttgga ggatc

25

<210> 294
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized primer

<400> 294
caccatggac acactttgct cacac

25

<210> 295
<211> 26
<212> DNA
<213> Artificial

<220>
<223> chemically synthesized primer

<400> 295
caccatggaa gttggggctg agctgg

26

<210> 296
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized primer

<400> 296
caccatggag ttgtggactg agctgg

26

<210> 297
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized primer

<400> 297
caccatggag tttgggctgt agctgg

26

<210> 298
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized primer

<400> 298
caccatggaa ctggggctcc gctgg

25

<210> 299

<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized primer

<400> 299
caccatggag ttggggctgt gctgg 25

<210> 300
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized primer

<400> 300
caccatggag ttttggctga gctgg 25

<210> 301
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized primer

<400> 301
caccatgacg gagtttgggc tgagc 25

<210> 302
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized primer

<400> 302
caccatgaaa gcacctgtgg ttcttc 26

<210> 303
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized primer

<400> 303
caccatgaaa catctgtggt tcttc 25

<210> 304
<211> 25

<212> DNA
 <213> Artificial Sequence

 <220>
 <223> chemically synthesized primer

 <400> 304
 caccatgggg tcaaccgcca tcctc 25

<210> 305
 <211> 28
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> chemically synthesized primer

 <400> 305
 caccatgtct gtctccttcc tcctcttc 28

<210> 306
 <211> 21
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> chemically synthesized primer

 <400> 306
 atgggggtccc aggttcacct c 21

<210> 307
 <211> 22
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> chemically synthesized primer

 <400> 307
 atgttgccat cacaactcat tg 22

<210> 308
 <211> 98
 <212> PRT
 <213> Homo sapiens

 <400> 308
 Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
 20 25 30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

<210> 309
<211> 369
<212> DNA
<213> Homo sapiens

<400> 309
caggtgcagc tgggtgcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc 60
tcctgcaagg cttctggata caccttcacc ggctactata tgcactgggt ggcacaggcc 120
cctggacaag ggcttgagtg gatgggatgg atcaacccta acagtgggtg cacaactat 180
gcacagaagt ttcaggacag ggtcaccatg accagggaca cgtccatcag cacagcctac 240
atggagctga gcaggctgag atctgacgac acggccgtgt attactgtgc gagagatttc 300
tttggttcgg ggagtctcct ctactttgac tactggggcc agggaaccct ggtcaccgtc 360
tcctcagcc 369

<210> 310
<211> 123
<212> PRT
<213> Homo sapiens

<400> 310

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe

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<210> 314
<211> 17
<212> PRT
<213> Homo sapiens
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<400> 314

Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe Gln
 1 5 10 15

Asp

<210> 315

<211> 32

<212> PRT

<213> Homo sapiens

<400> 315

Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr Met Glu
 1 5 10 15

Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg
 20 25 30

<210> 316

<211> 13

<212> PRT

<213> Homo sapiens

<400> 316

Asp Phe Phe Gly Ser Gly Ser Leu Leu Tyr Phe Asp Tyr
 1 5 10

<210> 317

<211> 12

<212> PRT

<213> Homo sapiens

<400> 317

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
 1 5 10

<210> 318

<211> 339

<212> DNA

<213> Homo sapiens

<400> 318

gatattgtga tgaccagac tccactctct ctgtccgtca cccctggaca gccggcctcc 60
 atctcctgca agtctagtca ggcctcctg catagtggg gaaagaccta tttgtattgg 120
 tacctgcaga ggccaggcca gcctccacag ctctgatct atgaagtttc caaccggttc 180
 tctggagtgc cagataggtt cagtggcagc gggtcaggga cagatttcac actgaaaatc 240

agccgggtgg aggcgtgagga tgttgggggtt tattactgca tgcaaagtat acaccttcg 300
ctcactttcg gcggaggagac caaggtggag atcaaacga 339

<210> 319
<211> 113
<212> PRT
<213> Homo sapiens

<400> 319

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Gly Gly Lys Thr Tyr Leu Tyr Trp Tyr Leu Gln Arg Pro Gly Gln Pro
35 40 45

Pro Gln Leu Leu Ile Tyr Glu Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ser
85 90 95

Ile His Leu Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 320
<211> 23
<212> PRT
<213> Homo sapiens

<400> 320

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys
20

<210> 321
<211> 16

<212> PRT
<213> Homo sapiens

<400> 321

Lys Ser Ser Gln Ser Leu Leu His Ser Gly Gly Lys Thr Tyr Leu Tyr
1 5 10 15

<210> 322
<211> 15
<212> PRT
<213> Homo sapiens

<400> 322

Trp Tyr Leu Gln Arg Pro Gly Gln Pro Pro Gln Leu Leu Ile Tyr
1 5 10 15

<210> 323
<211> 7
<212> PRT
<213> Homo sapiens

<400> 323

Glu Val Ser Asn Arg Phe Ser
1 5

<210> 324
<211> 32
<212> PRT
<213> Homo sapiens

<400> 324

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
20 25 30

<210> 325
<211> 9
<212> PRT
<213> Homo sapiens

<400> 325

Met Gln Ser Ile His Leu Pro Leu Thr
1 5

<210> 326
<211> 11
<212> PRT
<213> Homo sapiens

<400> 326

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
1 5 10

<210> 327

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically synthesized primer

<400> 327

gaattcagag ttaaaccttg ag

22

<210> 328

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically synthesized primer

<400> 328

caggaatctg atctgttacc ac

22

<210> 329

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically synthesized primer

<400> 329

ctgaccctac aagatgccaa gag

23

<210> 330

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically synthesized primer

<400> 330

atcatgcatt gcaacattta ttgatggag

29

<210> 331

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically synthesized primer

<400> 331

ttggcagatt gtctgtagcc

20

<210> 332

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically synthesized primer

<400> 332

aggcattgtg catgctgctt

20

<210> 333

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically synthesized primer

<400> 333

tattgaaagt gccgagatcc

20

<210> 334

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically synthesized primer

<400> 334

tgcaaggacc acagccatc

19

<210> 335

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically synthesized primer

<400> 335

tcaatggaac cttcagcctt a

21

<210> 336

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically synthesized primer

<400> 336
gaaggggtgg gttttgaag 19

<210> 337
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized primer

<400> 337
ctcactgtga aagctgcagc accag 25

<210> 338
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized primer

<400> 338
tcaatggaac cttcagcctt a 21

<210> 339
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized primer

<400> 339
gaaggggtgg gttttgaag 19

<210> 340
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized primer

<400> 340
ctcactgtga aagctgcagc accag 25

<210> 341
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized

<400> 341
tgatcagtaa ggatttcacc tctgtttgta 30

<210> 342
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized

<400> 342
accttgatcat gtaccatcaa taaagtaccc 30

<210> 343
<211> 12
<212> PRT
<213> Homo sapiens

<400> 343

Ala Thr Thr Leu Lys Ser Tyr Asp Ser Asn Thr Pro
1 5 10

<210> 344
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> chemically synthesized

<400> 344

Leu Ser Ala Asp Asp Ala Lys Lys Asp Ala Ala Lys Lys Asp Asp Ala
1 5 10 15

Lys Lys Asp Asp Ala Lys Lys Asp Leu
20 25

<210> 345
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized primer

<400> 345
tctcttctc ctgctactct ggctcccaga taccaccggt gaaatagtga tgacgcagtc 60

<210> 346
<211> 57
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized primer

<400> 346
ccggaattct tactatttgt catcatcgtc cttataatcg ctagctgagg agacggg 57

<210> 347
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized primer

<400> 347
acgcgtcgac ccaccatgga agccccagcg cagcttctct tcctcctgct actctggctc 60

<210> 348
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized primer

<400> 348
tctcttcctc ctgctactct ggctcccaga taccaccggg gaaatagtga tgacgcagtc 60

<210> 349
<211> 57
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized primer

<400> 349
ccggaattct tactatttgt catcatcgtc cttataatcg ctagctttca gctccag 57

<210> 350
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized primer

<400> 350
acgcgtcgac ccaccatgga agccccagcg cagcttctct tcctcctgct actctggctc 60

<210> 351
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
 <223> chemically synthesized primer

 <400> 351
 actctggctc ccagatacca ccggagaaat agtgatgacg cagtctccag ccacc 55

 <210> 352
 <211> 51
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> chemically synthesized primer

 <400> 352
 ccgctcgagc tatttgtcat catcgctcctt ataatctttc agtccagct t 51

 <210> 353
 <211> 74
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> chemically synthesized primer

 <400> 353
 tcttcgagc caccatggaa accccagcgc agcttctctt cctcctgcta ctctggctcc 60
 cagataccac cgga 74

 <210> 354
 <211> 852
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> chemically synthesized primer

 <400> 354
 atggaagccc cagcgcagct tctcttctc ctgtactct ggctcccaga taccaccggt 60
 gaaatagtga tgacgcagtc tccagccacc ctgtctgtgt ctccagggga aagagccacc 120
 ctctcctgca gggccagtca gagtgttgac aacaacttag tctggtacca gcagaaacct 180
 ggccaggctc ccaggctcct catctatggt gcatccacca gggccactgg tatcccagcc 240
 aggttcagtg gcagtgggtc tgggacagag ttcaactctca ccatcagtag tctgcagtct 300
 gaagattttg cagtttatta ctgtcagcag tataataact ggctccgtg gacgttcggc 360
 caagggacca aggtggaaat caaactttcc gcggacgatg cgaaaaagga tgctgcgaag 420
 aaagatgacg ctaagaaaga cgatgctaaa aaggacctgc aggtgcagct gcaggagtcg 480
 ggcccaggac tggatgaagc ttcacagacc ctgtccctca cctgcactgt ctctgggtggc 540
 tccatcagca gttttaatta ctactggagc tggatccgcc accaccagc gaagggcctg 600

gagtggattg ggtacatcta ttacagtggg agcacctact ccaaccggtc cctcaagagt 660
 cgagttacca tatcagtaga cacgtctaag aaccagttct cctgacgt gagctctgtg 720
 actgccgcgg acacggccgt gtattactgt gcgagagggt ataactggaa ctactttgac 780
 tactggggcc agggaaacct ggtcaccgtc tcctcagcta gcgattataa ggacgatgat 840
 gacaaatagt aa 852

<210> 355
 <211> 282
 <212> PRT
 <213> Homo sapiens

<400> 355

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Trp Leu Pro
 1 5 10 15

Asp Thr Thr Gly Glu Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser
 20 25 30

Val Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser
 35 40 45

Val Asp Asn Asn Leu Val Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
 50 55 60

Arg Leu Leu Ile Tyr Gly Ala Ser Thr Arg Ala Thr Gly Ile Pro Ala
 65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser
 85 90 95

Ser Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Asn
 100 105 110

Asn Trp Pro Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 115 120 125

Leu Ser Ala Asp Asp Ala Lys Lys Asp Ala Ala Lys Lys Asp Asp Ala
 130 135 140

Lys Lys Asp Asp Ala Lys Lys Asp Leu Gln Val Gln Leu Gln Glu Ser
 145 150 155 160

Gly Pro Gly Leu Val Lys Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr
 165 170 175

Val Ser Gly Gly Ser Ile Ser Ser Phe Asn Tyr Tyr Trp Ser Trp Ile
180 185 190

Arg His His Pro Gly Lys Gly Leu Glu Trp Ile Gly Tyr Ile Tyr Tyr
195 200 205

Ser Gly Ser Thr Tyr Ser Asn Pro Ser Leu Lys Ser Arg Val Thr Ile
210 215 220

Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Thr Leu Ser Ser Val
225 230 235 240

Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Tyr Asn Trp
245 250 255

Asn Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
260 265 270

Ala Ser Asp Tyr Lys Asp Asp Asp Asp Lys
275 280

<210> 356
<211> 1617
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically synthesized

<400> 356
atggaagccc cagcgagct tctcttcctc ctgctactct ggctcccaga taccaccggt 60
gaaatagtga tgacgcagtc tccagccacc ctgtctgtgt ctccagggga aagagccacc 120
ctctcctgca gggccagtc gagtggtgac aacaacttag tctggtacca gcagaaacct 180
ggccaggctc ccaggctcct catctatggt gcattccacca gggccactgg tatcccagcc 240
aggttcagtg gcagtgggtc tgggacagag ttcactctca ccatcagtag tctgcagttc 300
gaagattttg cagtttatta ctgtcagcag tataataact ggcctccgtg gacgttcggc 360
caagggacca aggtggaaat caaactttcc gcgagcagtg cgaaaaagga tgctgcgaag 420
aaagatgacg ctaagaaaga cgatgctaaa aaggacctgc aggtgcagct gcaggagtcg 480
ggcccaggac tggatgaagc ttacagacc ctgtccctca cctgcactgt ctctgggtggc 540
tccatcagca gttttaatta ctactggagc tggatccgcc accaccagga gaagggcctg 600
gagtggattg ggtacatcta ttacagtggg agcacctact ccaaccgctc cctcaagagt 660

cgagttacca tatcagtaga cacgtctaag aaccagttct ccctgacgct gagctctgtg 720
 actgccgcgg acacggccgt gtattactgt gcgagagggt ataactggaa ctactttgac 780
 tactggggcc agggaaacct ggtcaccgtc tcctcaggag gtgggtggatc cgatatcaaa 840
 ctgcagcagt caggggctga actggcaaga cctggggcct cagtgaagat gtcctgcaag 900
 acttctggct acacctttac taggtacacg atgcactggg taaaacagag gcctggacag 960
 ggtctggaat ggattggata cattaatcct agccgtgggt atactaatta caatcagaag 1020
 ttcaaggaca aggccacatt gactacagac aaatcctcca gcacagccta catgcaactg 1080
 agcagcctga catctgagga ctctgcagtc tattactgtg caagatatta tgatgatcat 1140
 tactgccttg actactgggg ccaaggcacc actctcacag tctcctcact ttccgcggac 1200
 gatgcgaaaa aggatgctgc gaagaaagat gacgctaaga aagacgatgc taaaaaggac 1260
 ctggacattc agctgaccca gtctccagca atcatgtctg catctccagg ggagaaggtc 1320
 accatgacct gcagagccag ttcaagtgtg agttacatga actggtacca gcagaagtca 1380
 ggcacctccc caaaagatg gatttatgac acatccaaag tggcttctgg agtcccttat 1440
 cgcttcagtg gcagtgggtc tgggacctca tactctctca caatcagcag catggaggct 1500
 gaagatgctg ccacttatta ctgccaacag tggagtagta acccgctcac gtccggtgct 1560
 gggaccaagc tggagctgaa agctagcgat tataaggacg atgatgacaa atagtaa 1617

<210> 357
 <211> 537
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> chemically synthesized
 <400> 357

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Trp Leu Pro
 1 5 10 15

Asp Thr Thr Gly Glu Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser
 20 25 30

Val Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser
 35 40 45

Val Asp Asn Asn Leu Val Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
 50 55 60

Arg Leu Leu Ile Tyr Gly Ala Ser Thr Arg Ala Thr Gly Ile Pro Ala
 65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Asn
100 105 110

Asn Trp Pro Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
115 120 125

Leu Ser Ala Asp Asp Ala Lys Lys Asp Ala Ala Lys Lys Asp Asp Ala
130 135 140

Lys Lys Asp Asp Ala Lys Lys Asp Leu Gln Val Gln Leu Gln Glu Ser
145 150 155 160

Gly Pro Gly Leu Val Lys Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr
165 170 175

Val Ser Gly Gly Ser Ile Ser Ser Phe Asn Tyr Tyr Trp Ser Trp Ile
180 185 190

Arg His His Pro Gly Lys Gly Leu Glu Trp Ile Gly Tyr Ile Tyr Tyr
195 200 205

Ser Gly Ser Thr Tyr Ser Asn Pro Ser Leu Lys Ser Arg Val Thr Ile
210 215 220

Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Thr Leu Ser Ser Val
225 230 235 240

Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Tyr Asn Trp
245 250 255

Asn Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
260 265 270

Gly Gly Gly Gly Ser Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu
275 280 285

Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr
290 295 300

Thr Phe Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln
305 310 315 320

Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn
 325 330 335
 Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser
 340 345 350
 Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser
 355 360 365
 Ala Val Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp
 370 375 380
 Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Leu Ser Ala Asp
 385 390 395 400
 Asp Ala Lys Lys Asp Ala Ala Lys Lys Asp Asp Ala Lys Lys Asp Asp
 405 410 415
 Ala Lys Lys Asp Leu Asp Ile Gln Leu Thr Gln Ser Pro Ala Ile Met
 420 425 430
 Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser
 435 440 445
 Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro
 450 455 460
 Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser Gly Val Pro Tyr
 465 470 475 480
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser
 485 490 495
 Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser
 500 505 510
 Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Ala
 515 520 525
 Ser Asp Tyr Lys Asp Asp Asp Asp Lys
 530 535

<210> 358
 <211> 1677
 <212> DNA
 <213> Artificial Sequence

<220>

<223> chemically synthesized

<400> 358

atggaaaccc cagcgcagct tctcttcctc ctgctactct ggctcccaga taccaccgga	60
gaaatagtga tgacgcagtc tccagccacc ctgtctgtgt ctccagggga aagagccacc	120
ctctcctgca gggccagtca gagtgttgac aacaacttag tctggtacca gcagaaacct	180
ggccaggctc ccaggctcct catctatggt gcatccacca gggccactgg tatcccagcc	240
aggttcagtg gcagtgggtc tgggacagag ttactctca ccatcagtag tctgcagtct	300
gaagattttg cagtttatta ctgtcagcag tataataact ggcctccgtg gacgttcggc	360
caagggacca aggtggaaat caaactttcc gcggacgatg cgaaaaagga tgctgcgaag	420
aaagatgacg ctaagaaaga cgatgctaaa aaggacctgc aggtgcagct gcaggagtcg	480
ggcccaggac tggatgaagc ttacagacc ctgtccctca cctgcactgt ctctggtggc	540
tccatcagca gttttaatta ctactggagc tggatccgcc accaccagg gaagggcctg	600
gagtggattg ggtacatcta ttacagtggg agcacctact ccaaccctgc cctcaagagt	660
cgagttacca tatcagtaga cagctctaag aaccagttct ccctgacgct gagctctgtg	720
actgccgcgg acacggccgt gtattactgt gcgagagggt ataactggaa ctactttgac	780
tactggggcc agggaaacct ggtcacctgc tcctcattat cagcggatga cgccaagaaa	840
gacgcagcca aaaaggacga tgcaaagaag gatgacgcaa agaaagattt agatatcaaa	900
ctgcagcagt caggggctga actggcaaga cctggggcct cagtgaagat gtcctgcaag	960
acttctggct acacctttac taggtacacg atgcactggg taaaacagag gcctggacag	1020
ggtctggaat ggattggata cattaatcct agccgtgggt atactaatta caatcagaag	1080
ttcaaggaca aggccacatt gactacagac aaatcctcca gcacagccta catgcaactg	1140
agcagcctga catctgagga ctctgcagtc tattactgtg caagatatta tgatgatcat	1200
tactgccttg actactgggg ccaaggcacc actctcacag tctcctcact ttccgcggac	1260
gatgcgaaaa aggatgctgc gaagaaagat gacgctaaga aagacgatgc taaaaaggac	1320
ctggacattc agctgaccca gtctccagca atcatgtctg catctccagg ggagaaggtc	1380
accatgacct gcagagccag ttcaagtgtg agttacatga actggtacca gcagaagtca	1440
ggcaacctcc ccaaaagatg gatttatgac acatccaaag tggcttctgg agtccttat	1500
cgcttcagtg gcagtgggtc tgggacctca tactctctca caatcagcag catggaggct	1560
gaagatgctg ccacttatta ctgccaacag tggagtagta acccgctcac gttcgggtgt	1620
gggaccaagc tggagctgaa agattataag gacgatgatg acaaatagct cgagcgg	1677

<210> 359
 <211> 555
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> chemically synthesized

<400> 359

Met Glu Thr Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
 1 5 10 15

Asp Thr Thr Gly Glu Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser
 20 25 30

Val Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser
 35 40 45

Val Asp Asn Asn Leu Val Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
 50 55 60

Arg Leu Leu Ile Tyr Gly Ala Ser Thr Arg Ala Thr Gly Ile Pro Ala
 65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser
 85 90 95

Ser Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Asn
 100 105 110

Asn Trp Pro Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 115 120 125

Leu Ser Ala Asp Asp Ala Lys Lys Asp Ala Ala Lys Lys Asp Asp Ala
 130 135 140

Lys Lys Asp Asp Ala Lys Lys Asp Leu Gln Val Gln Leu Gln Glu Ser
 145 150 155 160

Gly Pro Gly Leu Val Lys Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr
 165 170 175

Val Ser Gly Gly Ser Ile Ser Ser Phe Asn Tyr Tyr Trp Ser Trp Ile
 180 185 190

Arg His His Pro Gly Lys Gly Leu Glu Trp Ile Gly Tyr Ile Tyr Tyr
 195 200 205

Ser Gly Ser Thr Tyr Ser Asn Pro Ser Leu Lys Ser Arg Val Thr Ile.
 210 215 220

Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Thr Leu Ser Ser Val
 225 230 235 240

Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Tyr Asn Trp
 245 250 255

Asn Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 260 265 270

Leu Ser Ala Asp Asp Ala Lys Lys Asp Ala Ala Lys Lys Asp Asp Ala
 275 280 285

Lys Lys Asp Asp Ala Lys Lys Asp Leu Asp Ile Lys Leu Gln Gln Ser
 290 295 300

Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys Lys
 305 310 315 320

Thr Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Lys Gln
 325 330 335

Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg
 340 345 350

Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr
 355 360 365

Thr Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr
 370 375 380

Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His
 385 390 395 400

Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
 405 410 415

Leu Ser Ala Asp Asp Ala Lys Lys Asp Ala Ala Lys Lys Asp Asp Ala
 420 425 430

Lys Lys Asp Asp Ala Lys Lys Asp Leu Asp Ile Gln Leu Thr Gln Ser
 435 440 445

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys
 450 455 460

Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser
 465 470 475 480

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser
 485 490 495

Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser
 500 505 510

Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys
 515 520 525

Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu
 530 535 540

Glu Leu Lys Asp Tyr Lys Asp Asp Asp Asp Lys
 545 550 555

<210> 360
 <211> 100
 <212> PRT
 <213> Homo sapiens

<400> 360

Gln Ile Thr Leu Lys Glu Ser Gly Pro Thr Leu Val Lys Pro Thr Gln
 1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
 20 25 30

Gly Val Gly Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
 35 40 45

Trp Leu Ala Leu Ile Tyr Trp Asn Asp Asp Lys Arg Tyr Ser Pro Ser
 50 55 60

Leu Lys Ser Arg Leu Thr Ile Thr Lys Asp Thr Ser Lys Asn Gln Val
 65 70 75 80

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
 85 90 95

Cys Ala His Arg

100

<210> 361
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 361

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
 20 25 30

Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 362
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 362

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ser Ile Ser Ser Ser Ser Ser Tyr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 363
 <211> 98
 <212> PRT
 <213> Homo sapiens
 <400> 363

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

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Trp Ile Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
 50 55 60

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Ser Pro Gln Leu Leu Ile Tyr Thr Leu Ser Tyr Arg Ala Ser Gly Val
 50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys
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Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
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Tyr Gly Ala Ser Thr Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
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 35 40 45

Trp Ile Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Tyr Ser Asn Pro Ser
 50 55 60

Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
 65 70 75 80

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 85 90 95

Cys Ala Arg Gly Tyr Asn Trp Asn Tyr Phe Asp Tyr Trp Gly Gln Gly
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Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
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Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
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Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
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Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
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 Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
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 Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
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 35 40 45

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420 425 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

Lys

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 A61P 35/00 (2006.01) C12N 5/10 (2006.01)
 C07K 16/30 (2006.01) G01N 33/574 (2006.01)
 C07K 19/00 (2006.01) G01N 33/577 (2006.01)
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 Filed on 7 November 2005 (07.11.2005)

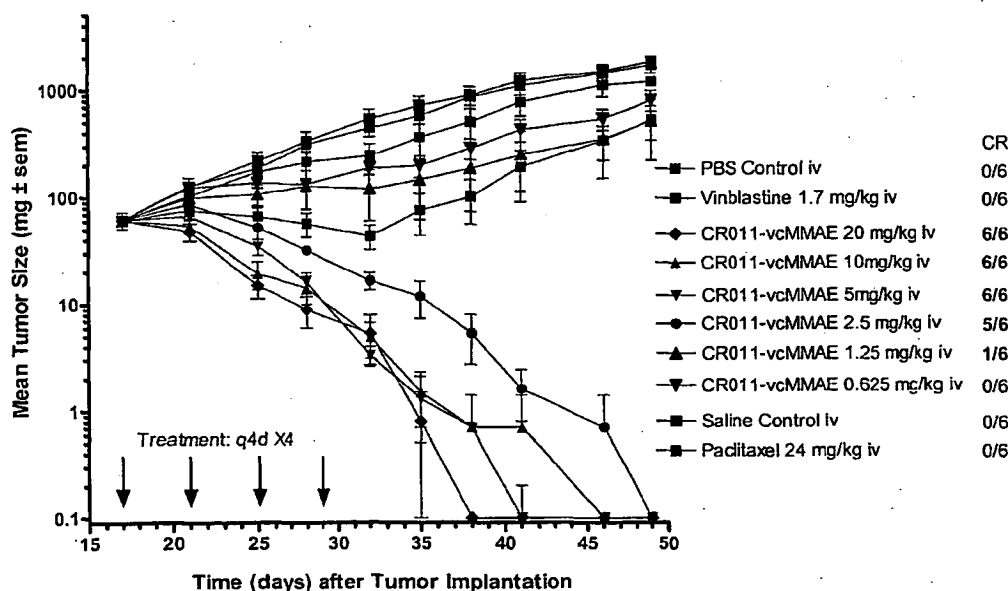
(71) Applicants (for all designated States except US): CURAGEN CORPORATION [US/US]; 322 East Main Street, Branford, CT 06405 (US). ABGENIX, INC. [US/US]; 6701 Kaiser Drive, Fremont, CA 94555 (US).

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[Continued on next page]

(54) Title: ANTIBODIES DIRECTED TO GPNMB AND USES THEREOF



(57) Abstract: The present invention provides fully human monoclonal antibodies that specifically bind to GPNMB, and uses thereof. Nucleotide sequences encoding, and amino acid sequences comprising, heavy and light chain immunoglobulin molecules, particularly sequences corresponding to contiguous heavy and light chain sequences spanning the framework regions and/or complementarity determining regions (CDRs) are provided. The present invention also provides immunoconjugates comprising anti-GPNMB antibodies and methods of using such immunoconjugates. The present invention further provides bispecific antibodies comprising an anti-GPNMB antibody component and an anti-CD3 component, and methods of using such bispecific antibodies.



CT 06443 (US). **ARA, Gulshan** [US/US]; 40 Sunny-side Drive, Northford, CT 06472 (US). **MEZES, Peter** [CA/US]; 7 Clark's Lane, Old Lyme, CT 06371 (US). **CHAPOVAL, Andrei** [RU/US]; 135 Versailles Cir. Apt. B, Towson, MD 21204 (US).

(74) Agent: **ELRIFI, Ivor, R.**; Mintz, Levin, Cohn, Ferris, Glovsky and Popeo PC, One Financial Center, Boston, MA 02111 (US).

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG,

SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US (patent), UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

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International application No

PCT/US2005/043482

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/395 A61K47/48 A61P35/00 C07K16/30 C07K19/00
C12N1/21 C12N15/13 C12N15/63 C12N5/10 G01N33/574
G01N33/577

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C07K C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	KAM FAI TSE E AL.: "CR011, a potent fully human monoclonal antibody-auristatin E conjugated prodrug targeting melanoma" 96TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH IN ANAHEIM, CA. ABSTRACT 557, 16 April 2005 (2005-04-16), XP002403962 HTTP://WWW.CURAGEN.COM/AACR-EORTC-NCI_2005 POSTERS.PDF abstract ----- -/-	1-45

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

23 October 2006

Date of mailing of the international search report

22/11/2006

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
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Authorized officer

Cilensek, Zoran

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2005/043482

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KUAN CHIEN-TSUN ET AL: "Monoclonal antibodies recognizing human gpnmbwt/gpnmbv react with human high-grade gliomas (HGL)." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, vol. 44, July 2003 (2003-07), pages 1116-1117, XP008070220 & 94TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH; WASHINGTON, DC, USA; JULY 11-14, 2003 ISSN: 0197-016X abstract	1-13, 15-34, 37-45
T	TSE KAM FAI ET AL: "CR011, a fully human monoclonal antibody-auristatin E conjugate, for the treatment of melanoma." CLINICAL CANCER RESEARCH : AN OFFICIAL JOURNAL OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH. 15 FEB 2006, vol. 12, no. 4, 15 February 2006 (2006-02-15), pages 1373-1382, XP002403963 ISSN: 1078-0432	1-45
A	US 2002/102264 A1 (CHEUNG NAI-KONG V [US]) 1 August 2002 (2002-08-01) page 9, column 1, paragraph 3 page 19, column 1, paragraph 4 page 57, column 2, paragraph 3	1-45

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/043482

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 37-43 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2005/043482

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2002102264	A1	01-08-2002	NONE